	L #	Hits	Search Text	DBs	Time Stamp
1	L1	88	hbx	USPAT; US-PGPUB	2002/10/02 12:30
2	L2	302	(hepatitis adj b adj virus or hbv) near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:31
3	L3	12	1 and 2	USPAT; US-PGPUB	2002/10/02 12:31
4	L4	5	hbx near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:35
5	L5	5066	src	USPAT; US-PGPUB	2002/10/02 12:36
6	L6	583665	activat$8	USPAT; US-PGPUB	2002/10/02 12:37
7	L7	176893	upstream	USPAT; US-PGPUB	2002/10/02 12:38
8	L8	2125	6 near5 7	USPAT; US-PGPUB	2002/10/02 12:39
9	L9	12	8 same 5	USPAT; US-PGPUB	2002/10/02 12:39
10	L10	31	5 same 6 same 7	USPAT; US-PGPUB	2002/10/02 14:35
11	L11	145	5 near2 (activator$1 or activation)	USPAT; US-PGPUB	2002/10/02 14:45
12	L12	2228	hbv or hbx	USPAT; US-PGPUB	2002/10/02 14:45
13	L13	5	11 and 12	USPAT; US-PGPUB	2002/10/02 14:45
14	L14	365	5 near5 6	USPAT; US-PGPUB	2002/10/02 14:54
15	L15	5	12 and 13	USPAT; US-PGPUB	2002/10/02 14:56
16	L16	15	12 and 14	USPAT; US-PGPUB	2002/10/02 14:56

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	88	hbx	USPAT; US-PGPUB	2002/10/02 12:30
2	L2	302	(hepatitis adj b adj virus or hbv) near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:31
3	L3	12	1 and 2	USPAT; US-PGPUB	2002/10/02 12:31
4	L4	5	hbx near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:35

PGPUB-DOCUMENT-NUMBER: 20020045191

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020045191 A1

TITLE: Inhibition of the SRC kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

PUBLICATION-DATE: April 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Schneider, Robert J.	New York	NY	US	
Klein, Nicola	Palo Alto	CA	US	

US-CL-CURRENT: 435/7.1;435/15 ;514/262.1 ;514/44 ;514/520 ;514/7 ;536/24.5

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

US-PAT-NO: 6420338

DOCUMENT-IDENTIFIER: US 6420338 B1

TITLE: Inhibition of the Src kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

DATE-ISSUED: July 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schneider; Robert J.	New York	NY	N/A	N/A
Klein; Nicola	New York	NY	N/A	N/A

US-CL-CURRENT: 514/12; 514/262.1 ; 514/451 ; 514/619 ; 514/646 ; 514/789

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

9 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	88	hbx	USPAT; US-PGPUB	2002/10/02 12:30
2	L2	302	(hepatitis adj b adj virus or hbv) near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:31
3	L3	12	1 and 2	USPAT; US-PGPUB	2002/10/02 12:31
4	L4	5	hbx near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:35

PGPUB-DOCUMENT-NUMBER: 20020045191

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020045191 A1

TITLE: Inhibition of the SRC kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

PUBLICATION-DATE: April 18, 2002

INVENTOR-INFORMATION:
NAME CITY STATE COUNTRY RULE-47
Schneider, Robert J. New York NY US
Klein, Nicola Palo Alto CA US

APPL-NO: 09/ 955006

DATE FILED: September 17, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60232892 20000915 US

US-CL-CURRENT: 435/7.1,435/15 ,514/262.1 ,514/44 ,514/520 ,514/7 ,536/24.5

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX:

[0014] The present invention relates to the treatment and revention of HBV infection by targeting activation of the Src family of kinases. The present invention further relates to the treatment and prevention of HBV infection by

targeting activation of cytosolic calcium release and Pyk2-Src signal transduction. The present invention also relates to compounds which inhibit HBx-mediated activation of the Pyk2 tyrosine kinase and Src family of kinases as well as the downstream components of the Pyk2-Src kinase signaling cascade for the treatment of HBV infection.

Summary of Invention Paragraph - BSTX:

[0017] The present invention encompasses a variety of techniques and compounds to target the activities of HBx essential for HBV replication. In particular, these include, but are not limited to HBx-mediated activation of the Src kinase family signal transduction pathways for the treatment and prevention of HBV infection. The present invention encompasses the use of known inhibitors of Pyk2 tyrosine kinase signal transduction, in addition to inhibitors of calcium channels and their regulatory components, to treat HBV infection. The invention encompasses the use of known Src inhibitors to treat HBV infection. Examples of such specific inhibitors include, but not limited to: Pyk2 specific tyrosine kinase inhibitors, Src specific tyrosine kinase inhibitors, such as CsK, tyrphostin-derived inhibitors, derivatives of benzylidenemalonitrile, pyrazolopyrimidine PP1, and microbial agents, such as angelmicin B; and competitive inhibitors, such as small phosphotyrosine containing ligands. The invention also encompasses the use of known HBx inhibitors for the treatment of HBV, including, but not limited to, antisense RNAs directed to HBx. The present invention also relates to the use of inhibitors of downstream effectors of Src kinases, including but not limited to, cytoplasmic factors, such as Ras, Raf, focal adhesion kinase (FAK) and MAPK, and nuclear factors, such as Myc and cyclin-dependent kinases.

Summary of Invention Paragraph - BSTX:

[0019] The present invention further relates to screening assays to identify compounds which inhibit HBx-mediated activation of the Src kinase signaling pathway and may be used to treat HBV infection and diseases and disorders associated with HBV infection. The present invention also relates to screening assays to identify compounds which inhibit HBx activation of Pyk2 tyrosine kinase and their regulatory components and may be used to treat HBV infection and diseases and disorders associated with HBV infection.

Detail Description Paragraph - DETX:

[0052] The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx-mediated activation of calcium-dependent tyrosine kinase, PhK2, HBx-mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target cytosolic calcium release, regulation of calcium channels and thus, inhibit HBx-mediated activation of calcium-dependent tyrosine kinase Pyk2. The invention further relates to pharmaceutical compositions for the treatment of

HBV-infection targeted to HBx and its essential activities required to sustain HBV replication.

Detail Description Paragraph - DETX:

[0054] The present invention encompasses a variety of protocols to inhibit HBV replication and infection, including but not limited to: (1) protocols which target and inhibit HBx expression or inhibit the essential activities of HBx which may lead to activation of the calcium-dependent tyrosine kinase Pyk2 and the Src kinase signaling cascades; (2) protocols which target and inhibit upstream effectors of the Src family of kinases, such as cytosolic calcium release, which may or may not be activated by HBx, but are required for activation of Pyk2-Src kinase signaling cascades; and (3) protocols which target and inhibit Pyk2 tyrosine kinases, Src kinase family members, Src-activated enzymes and downstream effectors of Src kinases and their signal transduction pathways that are essential for viral replication.

Detail Description Paragraph - DETX:

[0056] The present invention relates to cell-based and animal model based screening assays to identify novel anti-HBV agents which target HBx and its interaction and/or activation of cellular components of calcium channels, the calcium-dependent tyrosine kinase, Pyk2, and the Src kinase signaling cascade. In addition, the present invention relates to screening assays to identify novel antiviral agents which inhibit HBx mediated activation of Src kinase and/or downstream effectors of the Src kinase signaling cascade, such as the nuclear factor, Myc. A variety of protocols and techniques may be utilized to screen for agents which interfere with and/or inhibit the interaction and/or activation of cytosolic calcium release and the Pyk2-Src kinase signaling cascade by HBx.

Detail Description Paragraph - DETX:

[0066] The Applicants have further demonstrated that the expression of a Src inhibitor, i.e., the Csk protein, or dominant-negative (interfering or signaling incompetent) forms of Src and Fyn proteins resulted in the inhibition of HBx mediated activation of downstream components of the Src kinase signaling cascade. Activation of Src kinase initiates a number of downstream cascades of intracellular phosphorylation events. Activated Src results in activation of Ras, a prototypic member of the low-molecular weight family of protein GTPases which cycles between an inactive GDP-bound state and an active GTP-bound state. Activated Src also acts independently of the Ras signaling cascade to activate the nuclear factor Myc, among other proteins and kinases (reviewed in Erpel et al., 1995 supra). The formation of active Ras-GTP complexes controls a number of downstream cellular events, including opposing cellular processes, growth and differentiation (Boguski et al. 1993 Nature 366: 643-653). Active GTP-bound Ras associates and activates Raf. Activated Src has also been shown to bypass activation of Ras-GTP complexes to activate Raf in a Ras-independent manner (Stokoe & McCormick 1997 EMBOJ. 16:2384-2396). Activated Raf then phosphorylates and activates Mitogen-Activated Protein Kinase Kinase (MEK)

(Dent et al. 1992 Science 257:1404-1407; Howe et al. 1992 Cell 71:335-342), which in turn phosphorylates both tyrosines and threonines the extracellular-signal-reg- ulated protein Kinases (ERKs), members of the MAP kinase (MAPK) family.

Detail Description Paragraph - DETX:

[0067] Applicants have further demonstrated that the expression a Src inhibitors, i.e., Csk protein, or dominant-negative Src or Fyn proteins resulted in the inhibition of HBx activation of downstream components of Src kinase signaling cascade. Applicants have also shown that the expression of Src dominant-negative mutants, such as Csk, inhibited the ability of HBx to stimulate activities of the nuclear factor, Myc, including stimulation of cell cycle progression by blocking HBx activation of Src kinase signaling pathways. These findings clearly establish that activation of a Src kinase signaling cascade by HBx has a critical role in the hepadnaviral life cycle.

Detail Description Paragraph - DETX:

[0070] 5.2 Treatment of HBV-infection using Inhibitors of HBx Mediated Src Activation

Detail Description Paragraph - DETX:

[0074] For example, but not by way of limitation, compounds which may be used in accordance with the present invention encompass any compound which targets HBx and inhibits its expression or interferes with its activities required for HBV replication, including but not limited to dominant-negative mutants, antisense molecules and SELEX RNAs directed to HBx. The present invention further relates to nucleotides, peptides, polypeptides, fusion proteins and other compounds which further modulate HBx activities. Other examples of compounds include, but are not limited to peptide or other compounds, including small organic and inorganic molecules directed to regions of the HBx protein that are required either directly or indirectly for HBx activation of Src signal cascades.

Detail Description Paragraph - DETX:

[0079] A variety of techniques and compositions may be utilized to target Src kinase to inhibit its activity or to inhibit HBx mediated activation of components of the Src kinase mediated signaling cascade, thereby inhibiting HBV replication. Such techniques and compositions may include, but are not limited to, gene therapy approaches, drugs, small organic molecules identified to inhibit Src kinase, Ras, Raf, MAPK kinase, MAPK, c-Myc, cyclin-dependent kinases and/or other downstream effectors of the Src kinase signaling cascade.

Detail Description Paragraph - DETX:

[0150] Alternately, cell lines which co-express HBx and Src kinase and components of the Src kinase signaling cascade may be genetically engineered to assay for inhibitors of HBx activation of Src. This can be engineered in cell in the absence of HBV replication or in cell lines which support the HBV life cycle as a means of (1) identifying additional factors required to support the HBV life cycle, and (2) as a system to screen test compounds, for their ability to interfere with HBx activation and/or interaction with the Src kinase, and (3) as a system to screen test compounds for their ability to inhibit Src kinase activity and therefore inhibit HBV replication.

Detail Description Paragraph - DETX:

[0155] In yet another embodiment of the invention, the ability of a test agent to inhibit HBx activation of Src's induction of downstream effectors may be measured. For example, activation of Src kinase signaling cascade leads to enhanced expression of Myc proteins. Therefore, activation of Myc promoter elements may be used to determine the potential anti-HBV activity of the test agent. Constructs encoding the Myc promoter region linked to any of a variety of different reporter genes may be introduced into cells expressing the Src kinase and/or components of the Src kinase signaling cascade. Such reporter genes may include but is not limited to chloramphenicol acetyltransferase (CAT), luciferase, GUS, growth hormone, or placental alkaline phosphatase (SEAP). Following exposure of the cells to the test compound, the level of reporter gene expression may be quantitated to determine the test compound's ability to regulate receptor activity. Alkaline phosphatase assays are particularly useful in the practice of the invention as the enzyme is secreted from the cell. Therefore, tissue culture supernatant may be assayed for secreted alkaline phosphatase. In addition, alkaline phosphatase activity may be measured by calorimetric, bioluminescent or chemilumenscent assays such as those described in Bronstein, I. et al. (1994, Biotechniques 17: 172-177). Such assays provide a simple, sensitive easily automatable detection system for pharmaceutical screening.

Detail Description Paragraph - DETX:

[0158] In another embodiment of the cell-based assays of the present invention, activation of Src kinase signaling cascades mediated by HBx may be measured by a viability assay to positively test for effective compounds. For example, a test compound may be applied to cells expressing HBx and Src kinase signaling components. Then an agent which induces cell death in response-to activated Src kinase, such as tumor necrosis factor (TNF), is applied to cells. If the test compound is ineffective in inhibiting HBx mediated activation of Src kinase, the cells die. Compounds effective in inhibiting activation, result in cell viability. Such an assay system provides a quick and easy read-out to determine the effectiveness of a test compound to inhibit HBx mediated activation of Src kinase.

Detail Description Paragraph - DETX:

[0159] Alternatively, activation of Src kinase signaling pathways mediated by

HBx may be measured by the secretion of mature HBV viral particles into the medium of growing Chang cells. For example, Chang liver cells may be stably transformed with an HBV or WHV pregenome, or with a head-to-tail dimer of either HBV or WHV genomes. The integrated virus will produce and secrete HBV/WHV particles into the medium. As demonstrated by the Applicants, the secretion of viral particles is strongly enhanced by HBx protein activation of Src kinases. If the test compound is effective in inhibiting HBx activation of Src, it will result in reduced secretion of HBV/WHV particles into the medium. The level of particle secreted into the medium can be assayed using commercial ELISA kits to detect the presence of HBV/WHV HBcAg and HBsAg.

Detail Description Paragraph - DETX:

[0160] Alternatively, the activation of Src kinase signaling pathways mediated by HBx may be measured in fission yeast, Schizosaccharomyces pombe. Src kinase have not been found in single cell lower eukaroytes such as yeast, and their expression induces cell death of the fission yeast (Erpel, T., Superti-Furga, G. & Courtneidge, S. A., 1995, EMBO J. 14:963-975). Studies have shown that yeast cell viability is maintained only by inhibition of Src activation, for instance, by coexpression with the Src inhibitor Csk (Koegl, M., Courtneidge, S. A. & Superti-Furga, G. 1996, Oncogene 11:2317-2329). Therefore, a viability assay is based on the fact that activation of Src kinase by HBx protein in yeast will result in cell death, whereas the inhibition of Src kinase will permit cells to grow and reproduce. S. pombe can be transformed with the c-Src gene and the HBx gene under the control of regulated promoters. A variety of regulated promoters can be chosen, such as the thiamine repressible nmt 1 promoter. Removal of thiamine from the medium will result in induction of both HBx and c-Src proteins, and subsequent killing of cells. If a test compound is effective in inhibiting HBx activation of Src, it will block the growth arrest.

Detail Description Paragraph - DETX:

[0167] In yet another embodiment of the animal model screens of the present invention, the effect of test compounds to inhibit HBV replication may be measured indirectly. For example, transgenic mice may be engineered which express (1) the HBx gene product under the control of an inducible promoter, and (2) readout vector which is responsive to Src activation. The readout vector may comprise a reporter gene under the control of a Myc promoter. Such reporter constructs are described in Section 5.5.1 infra. In this assay system, expression of the HBx gene product is induced and the test compound is administered to the mice. The ability of the test compound to inhibit HBx mediated activation of Src kinase and HBV replication is assayed by measuring the reporter gene. Such reporter genes may include but are not limited to chloramphenicol acetyltransferase (CAT), luciferase, GUS, growth hormone, or placental alkaline phosphatase (SEAP). Following exposure of the animal to the test compound, the level of reporter gene expression may be quantitated from the blood or tissue sample to determine the test compound's ability to regulate receptor activity. Alkaline phosphatase assays are particularly useful in the practice of the invention as the enzyme is secreted from the cell. Therefore, tissue culture supernatant may be assayed for secreted alkaline phosphatase. In addition, alkaline phosphatase activity may be measured by calorimetric,

bioluminescent or chemilumenscent assays such as those described in Bronstein, I. et al. (1994, Biotechniques 17: 172-177). Such assays provide a simple, sensitive easily detection system for pharmaceutical screening.

Detail Description Paragraph - DETX:

[0192] Src family kinases were shown to be negatively regulated by phosphorylation of a regulatory carboxy-terminal tyrosine by the action of the protein tyrosine kinase C-terminal Src kinase (Csk). Csk specifically phosphorylates the carboxy-terminal tyrosine and returns Src to an inactive state. Therefore, overexpression of Csk can be used as a mechanism to negatively regulate Src and potentially inhibit the ability of HBx to activate the Ras cascade. To test whether HBx activation of the Src family of kinases is required for stimulation of Ras signaling by HBx, Chang cells were transfected at low density with plasmids expressing HBxo, HBx or HBx and Csk, serum-starved for 20 hours, 2% serum restored for 2 hours to permit protein synthesis, and activation of immunoprecipitated MAP kinase at the end of the Ras-MAP kinase cascade assayed by incubation with myelin basic protein (MBP) and [.gamma.-.sup.32P]ATP (FIG. 2). Expression of HBx induces a strong activation in the ability of MAP kinase to phosphorylate MBP that is abolished upon co-transfection of HBx with Csk. In contrast, overexpression of Csk only slightly impaired activation of MAP kinase by insulin, indicating that expression of Csk did not generally impair activation of the Ras signaling cascade. Thus, these data clearly demonstrate that HBx requires activation of the Src family of kinases for induction of the Ras signaling cascade.

Detail Description Paragraph - DETX:

[0226] The following experiments were conducted to address the question of whether HBx acts on the calcium-Pyk2 pathway, and if so, whether this is important for HBx stimulation of transcription and/or viral DNA replication. Three hepatic cell lines were transfected with vectors expressing HBx protein and transcription factor AP-1 dependent luciferase reporter (FIG. 8A). HBx induced a 3 to 6 fold increase in AP-1 directed transcription in all cell lines. Cotransfection of a dominant-inhibiting Pyk2 known as PKM (DiKic et al., 1996, Nature 383: 547-550), prevented activation of AP-1. Control studies showed that PKM decreased HBx expression from the CMV promoter by half. Since transfection of cells with 10 fold less HBx expression plasmid only reduced activation of AP-1 by 1.8 fold (FIG. 8A), these data indicate that PKM acts by inhibiting HBx activity rather than expression.

Detail Description Paragraph - DETX:

[0228] Studies determined whether HBx acts on intracellular calcium to activate Pyk2. HBx transfected Chang cells showed 5 fold increased phosphorylation of Pyk2 at Y-402, similar to TPA stimulation (FIG. 9A). Treatment with the cell permeable cytosolic calcium chelator BABTA-AM at 50 .mu.M (2 times IC.sub.50) for 2 h prevented Pyk2 phosphorylation without altering Pyk2 levels (FIG. 9A). HBx activation of Pyk2 therefore involves cytosolic calcium action. Studies next determined whether HBx acts on calcium channels in the endoplasmic

reticulum (ER), mitochondria or plasma membrane (PM) for its activity. A low (0.5 mM) concentration of EGTA was added to the culture medium for 2 h to block entrance of extracellular calcium (Zwick et al. 1999, J.B.C. 274: 20989-20996), or cells were treated with BAPTA-AM to block ER and mitochondrial calcium, or cyclosporin A (CsA) to block mitochondrial calcium function. EGTA had no effect whereas BAPTA-AM or GsA both prevented HBx activation of Pyk2, indicating that HBx acts on ERimitochondrial calcium control. A high concentration of EGTA (3 mM) did not block TPA activation of Pyk2 phosphorylation (Zwick et al. 1999, J.B.C. 274: 20989-20996) (FIG. 9C), but partially inhibited activation by HBx. Therefore, HBx acts on the control of ER/mitochondrial calcium, with low level entry of extracellular calcium, suggestive of constitutive cytosolic calcium alteration (Clapham 1997, Cell 80:259-268).

Detail Description Paragraph - DETX:

[0230] The requirement for cytosolic calcium in HBx-dependent viral replication was investigated. Cells transfected with wild type or HBx(-) HBV genomic DNA were treated for 4 d with 1 or 3 p.g/ml of CsA to block mitochondrial calcium channels. There was no evidence for CsA toxicity during treatment. CsA reduced HBV DNA replication in cytoplasmic core particles by 15 fold compared to untreated controls (FIG. 10A), similar in magnitude to inhibition of Pyk2 or the absence of HBx expression. Northern mRNA analysis demonstrated a 2 fold reduction in pgRLNA and HBsAg mRNAs (FIG. 10A). To determine whether inhibiting cytosolic calcium and Pyk2 activity inhibits HBV DNA replication, HepG2 cells were transfected with HBV genomic DNA and treated with CsA, or cotransfected with PKM. Cytosolic core particles were purified and incubated with [.alpha.-.sup.32P]-dNTPs to examine endogenous HBV polymerase activity (FIG. 10B). In untreated controls, predominantly full-length double-strand DNA products were produced, indicative of pgRNA reverse transcription and DNA-dependent DNA synthesis. PKM inhibition of Pyk2 or treatment of cells with CsA prevented DNA replication by 7 and 12 fold respectively. Treatment of HBV genome transfected cells with low levels of BAPTA-AM for 4 d impaired viral DNA replication by 10 fold without strongly reducing HBV mRNA levels (FIG. 10C). Collectively, these data show that HBx activation of HBV reverse transcription and DNA replication involves alteration of cytosolic calcium and coupled activation of Pyk2. The requirement for cytosolic calcium in HBx transcriptional stimulation was investigated in HepG2 cells transfected with luciferase reporters controlled by transcription factor AP- 1or CREB, with or without treatment of cells by CsA (FIG. 10D). HBx activation of AP- 1 dependent transcription was impaired 2.5 fold by treatment of cells with 10 .mu.g/ml CsA. HBx stimulation of CREB-dependent transcription was resistant to high dose CsA treatment, consistent with HBx activation of CREB by direct interaction (Andrisani et al., 1999, J. Oncol. 15: 1-7). These data indicate that HBx transcriptional activation of AP- 1 but not CREB requires alteration of cytosolic calcium.

Claims Text - CLTX:

20. A pharmaceutical formulation for the treatment of HBx infection, comprising a compound that inhibits HBx mediated activation of a Pyk2 kinase

signaling cascade, mixed with a pharmaceutically acceptable carrier.

Claims Text - CLTX:

21. A pharmaceutical formulation for the treatment of HBV infection that inhibits the activities of the HBx gene product essential to sustain the HBV life cycle, mixed with a pharmaceutically acceptable carrier.

US-PAT-NO: 6420338

DOCUMENT-IDENTIFIER: US 6420338 B1

TITLE: Inhibition of the Src kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

DATE-ISSUED: July 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schneider; Robert J.	New York	NY	N/A	N/A
Klein; Nicola	New York	NY	N/A	N/A

APPL-NO: 08/ 874430

DATE FILED: June 13, 1997

US-CL-CURRENT: 514/12; 514/262.1 ; 514/451 ; 514/619 ; 514/646 ; 514/789

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

9 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Brief Summary Text - BSTX:

The present invention relates to the treatment and prevention of HBV infection by targeting activation of the Src family of kinases. The present invention also relates to compounds which inhibit HBx-mediated activation of the Src

family of kinases as well as the downstream components of the Src kinase signaling cascade for the treatment of HBV infection.

Brief Summary Text - BSTX:

The present invention encompasses a variety of techniques and compounds to target the activities of HBx essential for HBV replication. In particular, these include, but are not limited to HBx-mediated activation of the Src kinase family signal transduction pathways for the treatment and prevention of HBV infection. The invention encompasses the use of known Src inhibitors to treat HBV infection. Examples of such specific inhibitors include, but not limited to: Src specific tyrosine kinase inhibitors, such as CsK, tyrphostin-derived inhibitors, derivatives of benzylidenemalonitrile, pyrazolopyrimidine PP1, and microbial agents, such as angelmicin B; and competitive inhibitors, such as small phosphotyrosine containing ligands. The invention also encompasses the use of known HBx inhibitors for the treatment of HBV, including, but not limited to, antisense RNAs directed to HBx. The present invention also relates to the use of inhibitors of downstream effectors of Src kinases, including but not limited to, cytoplasmic factors, such as Ras, Raf, focal adhesion kinase (FAK) and MAPK, and nuclear factors, such as Myc and cyclin-dependent kinases.

Detailed Description Text - DETX:

The present invention encompasses a variety of protocols to inhibit HBV replication and infection, including but not limited to: (1) protocols which target and inhibit HBx expression or inhibit the essential activities of HBx which may lead to activation of the Src kinase signaling cascades; (2) protocols which target and inhibit upstream effectors of the Src family of kinases, which may or may not be activated by HBx, but are required for activation of Src kinase signaling cascades; and (3) protocols which target and inhibit Src kinase family members, Src-activated enzymes and downstream effectors of Src kinases and their signal transduction pathways that are essential for viral replication.

Detailed Description Text - DETX:

The present invention relates to cell-based and animal model based screening assays to identify novel anti-HBV agents which target HBx and its interaction and/or activation of cellular components of the Src kinase signaling cascade. In addition, the present invention relates to screening assays to identify novel antiviral agents which inhibit HBx mediated activation of Src kinase and/or downstream effectors of the Src kinase signaling cascade, such as the nuclear factor, Myc. A variety of protocols and techniques may be utilized to screen for agents which interfere with and/or inhibit the interaction and/or activation of the Src kinase signaling cascade by HBX.

Detailed Description Text - DETX:

The Applicants have further demonstrated that the expression of a Src

inhibitor, i.e., the Csk protein, or dominant-negative (interfering or signaling incompetent) forms of Src and Fyn proteins resulted in the inhibition of HBx mediated activation of downstream components of the Src kinase signaling cascade. Activation of Src kinase initiates a number of downstream cascades of intracellular phosphorylation events. Activated Src results in activation of Ras, a prototypic member of the low-molecular weight family of protein GTPases which cycles between an inactive GDP-bound state and an active GTP-bound state. Activated Src also acts independently of the Ras signaling cascade to activate the nuclear factor Myc, among other proteins and kinases (reviewed in Erpel et al., 1995 supra). The formation of active Ras-GTP complexes controls a number of downstream cellular events, including opposing cellular processes, growth and differentiation (Boguski et al. 1993 Nature 366: 643-653). Active GTP-bound Ras associates and activates Raf. Activated Src has also been shown to bypass activation of Ras-GTP complexes to activate Raf in a Ras-independent manner (Stokoe & McCormick 1997 EMBO J. 16:2384-2396). Activated Raf then phosphorylates and activates Mitogen-Activated Protein Kinase Kinase (MEK) (Dent et al. 1992 Science 257:1404-1407; Howe et al. 1992 Cell 71:335-342), which in turn phosphorylates both tyrosines and threonines the extracellular-signal-regulated protein Kinases (ERKs), members of the MAP kinase (MAPK) family.

Detailed Description Text - DETX:

Applicants have further demonstrated that the expression a Src inhibitors, i.e., Csk protein, or dominant-negative Src or Fyn proteins resulted in the inhibition of HBx activation of downstream components of Src kinase signaling cascade. Applicants have also shown that the expression of Src dominant-negative mutants, such as Csk, inhibited the ability of HBx to stimulate activities of the nuclear factor, Myc, including stimulation of cell cycle progression by blocking HBx activation of Src kinase signaling pathways. These findings clearly establish that activation of a Src kinase signaling cascade by HBx has a critical role in the hepadnaviral life cycle.

Detailed Description Text - DETX:

5.2 Treatment of HBV-Infection Using Inhibitors of HBx Medicated Src Activation

Detailed Description Text - DETX:

For example, but not by way of limitation, compounds which may be used in accordance with the present invention encompass any compound which targets HBx and inhibits its expression or interferes with its activities required for HBV replication, including but not limited to dominant-negative mutants, antisense molecules and SELEX RNAs directed to HBx. The present invention further relates to nucleotides, peptides, polypeptides, fusion proteins and other compounds which further modulate HBx activities. Other examples of compounds include, but are not limited to peptide or other compounds, including small organic and inorganic molecules directed to regions of the HBx protein that are required either directly or indirectly for HBx activation of Src signal cascades.

Detailed Description Text - DETX:

A variety of techniques and compositions may be utilized to target Src kinase to inhibit its activity or to inhibit HBx mediated activation of components of the Src kinase mediated signaling cascade, thereby inhibiting HBV replication. Such techniques and compositions may include, but are not limited to, gene therapy approaches, drugs, small organic molecules identified to inhibit Src kinase, Ras, Raf, MAPK kinase, MAPK, c-Myc, cyclin-dependent kinases and/or other downstream effectors of the Src kinase signaling cascade.

Detailed Description Text - DETX:

Alternately, cell lines which co-express HBx and Src kinase and components of the Src kinase signaling cascade may be genetically engineered to assay for inhibitors of HBx activation of Src. This can be engineered in cell in the absence of HBV replication or in cell lines which support the HBV life cycle as a means of (1) identifying additional factors required to support the HBV life cycle, and (2) as a system to screen test compounds, for their ability to interfere with HBx activation and/or interaction with the Src kinase, and (3) as a system to screen test compounds for their ability to inhibit Src kinase activity and therefore inhibit HBV replication.

Detailed Description Text - DETX:

In yet another embodiment of the invention, the ability of a test agent to inhibit HBx activation of Src's induction of downstream effectors may be measured. For example, activation of Src kinase signaling cascade leads to enhanced expression of Myc proteins. Therefore, activation of Myc promoter elements may be used to determine the potential anti-HBV activity of the test agent. Constructs encoding the Myc promoter region linked to any of a variety of different reporter genes may be introduced into cells expressing the Src kinase and/or components of the Src kinase signaling cascade. Such reporter genes may include but is not limited to chloramphenicol acetyltransferase (CAT), luciferase, GUS, growth hormone, or placental alkaline phosphatase (SEAP). Following exposure of the cells to the test compound, the level of reporter gene expression may be quantitated to determine the test compound's ability to regulate receptor activity. Alkaline phosphatase assays are particularly useful in the practice of the invention as the enzyme is secreted from the cell. Therefore, tissue culture supernatant may be assayed for secreted alkaline phosphatase. In addition, alkaline phosphatase activity may be measured by calorimetric, bioluminescent or chemilumenscent assays such as those described in Bronstein, I. et al. (1994, Biotechniques 17: 172-177). Such assays provide a simple, sensitive easily automatable detection system for pharmaceutical screening.

Detailed Description Text - DETX:

In another embodiment of the cell-based assays of the present invention,

activation of Src kinase signaling cascades mediated by HBx may be measured by a viability assay to positively test for effective compounds. For example, a test compound may be applied to cells expressing HBx and Src kinase signaling components. Then an agent which induces cell death in response to activated Src kinase, such as tumor necrosis factor (TNF), is applied to cells. If the test compound is ineffective in inhibiting HBx mediated activation of Src kinase, the cells die. Compounds effective in inhibiting activation, result in cell viability. Such an assay system provides a quick and easy read-out to determine the effectiveness of a test compound to inhibit HBx mediated activation of Src kinase.

Detailed Description Text - DETX:

Alternatively, activation of Src kinase signaling pathways mediated by HBx may be measured by the secretion of mature HBV viral particles into the medium of growing Chang cells. For example, Chang liver cells may be stably transformed with an HBV or WHV pregenome, or with a head-to-tail dimer of either HBV or WHV genomes. The integrated virus will produce and secrete HBV/WHV particles into the medium. As demonstrated by the Applicants, the secretion of viral particles is strongly enhanced by HBx protein activation of Src kinases. If the test compound is effective in inhibiting HBx activation of Src, it will result in reduced secretion of HBV/WHV particles into the medium. The level of particle secreted into the medium can be assayed using commercial ELISA kits to detect the presence of HBV/WHV HBcAg and HBsAg.

Detailed Description Text - DETX:

Alternatively, the activation of Src kinase signaling pathways mediated by HBx may be measured in fission yeast, Schizosaccharomyces pombe. Src kinase have not been found in single cell lower eukaroytes such as yeast, and their expression induces cell death of the fission yeast (Erpel, T., Superti-Furga, G. & Courtneidge, S. A., 1995, EMBO J. 14:963-975). Studies have shown that yeast cell viability is maintained only by inhibition of Src activation, for instance, by coexpression with the Src inhibitor Csk (Koegl, M., Courtneidge, S. A. & Superti-Furga, G. 1996, Oncogene 11:2317-2329). Therefore, a viability assay is based on the fact that activation of Src kinase by HBx protein in yeast will result in cell death, whereas the inhibition of Src kinase will permit cells to grow and reproduce. S. pombe can be transformed with the c-Src gene and the HBx gene under the control of regulated promoters. A variety of regulated promoters can be chosen, such as the thiamine repressible nmt 1 promoter. Removal of thiamine from the medium will result in induction of both HBx and c-Src proteins, and subsequent killing of cells. If a test compound is effective in inhibiting HBx activation of Src, it will block the growth arrest.

Detailed Description Text - DETX:

In yet another embodiment of the animal model screens of the present invention, the effect of test compounds to inhibit HBV-replication may be measured indirectly. For example, transgenic mice may be engineered which express (1) the HBx gene product under the control of an inducible promoter, and (2)

readout vector which is responsive to Src activation. The readout vector may comprise a reporter gene under the control of a Myc promoter. Such reporter constructs are described in Section 5.5.1 infra. In this assay system, expression of the HBx gene product is induced and the test compound is administered to the mice. The ability of the test compound to inhibit HBx mediated activation of Src kinase and HBV replication is assayed by measuring the reporter gene. Such reporter genes may include but are not limited to chloramphenicol acetyltransferase (CAT), luciferase, GUS, growth hormone, or placental alkaline phosphatase (SEAP). Following exposure of the animal to the test compound, the level of reporter gene expression may be quantitated from the blood or tissue sample to determine the test compound's ability to regulate receptor activity. Alkaline phosphatase assays are particularly useful in the practice of the invention as the enzyme is secreted from the cell. Therefore, tissue culture supernatant may be assayed for secreted alkaline phosphatase. In addition, alkaline phosphatase activity may be measured by calorimetric, bioluminescent or chemilumenscent assays such as those described in Bronstein, I. et al. (1994, Biotechniques 17: 172-177). Such assays provide a simple, sensitive easily detection system for pharmaceutical screening.

Detailed Description Text - DETX:

Src family kinases were shown to be negatively regulated by phosphorylation of a regulatory carboxy-terminal tyrosine by the action of the protein tyrosine kinase C-terminal Src kinase (Csk). Csk specifically phosphorylates the carboxy-terminal tyrosine and returns Src to an inactive state. Therefore, overexpression of Csk can be used as a mechanism to negatively regulate Src and potentially inhibit the ability of HBx to activate the Ras cascade. To test whether HBx activation of the Src family of kinases is required for stimulation of Ras signaling by HBx, Chang cells were transfected at low density: with plasmids expressing HBxo, HBx or HBx and Csk, serum-starved for 20 hours, 2% serum restored for 2 hours to permit protein synthesis, and activation of immunoprecipitated MAP kinase at the end of the Ras-MAP kinase cascade assayed by incubation with myelin basic protein (MBP) and [.gamma.-.sup.32 P]ATP (FIG. 2). Expression of HBx induces a strong activation in the ability of MAP kinase to phosphorylate MBP that is abolished upon co-transfection of HBx with Csk. In contrast, overexpression of Csk only slightly impaired activation of MAP kinase by insulin, indicating that expression of Csk did not generally impair activation of the Ras signaling cascade. Thus, these data clearly demonstrate that HBx requires activation of the Src family of kinases for induction of the Ras signaling cascade.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	88	hbx	USPAT; US-PGPUB	2002/10/02 12:30
2	L2	302	(hepatitis adj b adj virus or hbv) near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:31
3	L3	12	1 and 2	USPAT; US-PGPUB	2002/10/02 12:31
4	L4	5	hbx near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:35
5	L5	5066	src	USPAT; US-PGPUB	2002/10/02 12:36
6	L6	583665	activat$8	USPAT; US-PGPUB	2002/10/02 12:37
7	L7	176893	upstream	USPAT; US-PGPUB	2002/10/02 12:38
8	L8	2125	6 near5 7	USPAT; US-PGPUB	2002/10/02 12:39
9	L9	12	8 same 5	USPAT; US-PGPUB	2002/10/02 12:39

PGPUB-DOCUMENT-NUMBER: 20020137663

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137663 A1

TITLE: The anti-neoplastic agent ET-743 inhibits trans activation by SXR

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Forman, Barry M.	Newport Beach	CA	US	
Dussault, Isabelle	Thousand Oaks	CA	US	

APPL-NO: 09/ 927341

DATE FILED: August 13, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60224356 20000811 US

US-CL-CURRENT: 514/1

ABSTRACT:

ET-743 is a small molecular weight compound with antineoplastic activity that inhibits the ability of the nuclear receptor SXR to trans activate gene transcription from SXR regulated response elements. The nuclear receptor SXR has been identified as a receptor that activates transcription of the mdr1 gene and thus increases multidrug resistance in cells. The interaction of SXR with the mdr1 gene and ET-743 provide a set of physiological mechanisms which can be exploited to identify novel inhibitors of SXR activation and mdr1 gene transcription and thus novel agents which exhibit an antineoplastic effect against tumor cells either alone or when coadministered with another antineoplastic agent.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to provisional patent application Ser. No. 60/224,356, filed Aug. 11, 2000, and claims the benefit of the filing date of said provisional application.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0071] A mammalian two-hybrid assay was used to determine the effects of the Et-743 analog Pt650 on coregulator recruitment for SXR. CV-1 cells were transfected as indicated above with the indicated hybrid expression vectors and a .beta.-galactosidase vector as an internal control. Reporter activity was measured and normalized to the internal .beta.-galactosidase control and is reported as a proportion of internal .beta.-galactosidase activity. CV- 1 cells were transiently transfected with a GAL4 reporter construct and an expression vector encoding a first hybrid protein which is a DNA transcription activator containing the VP16 transactivation domain linked to the ligand binding domain of SXR (VP-L-SXR). In addition, cells were also transfected with expression vectors encoding the GAL4 DNA binding domain alone or a second hybrid protein which is the GAL4 DNA binding domain linked to the receptor interaction domains of the nuclear receptor coactivators **SRC** 1, ACTR, GRIP or PBP, or the nuclear receptor corepressors SMRT or NCoR, as indicated. The GAL4 reporter construct comprised four copies of a yeast GAL4 **upstream activation** sequence operatively linked to the herpes thymidine kinase promoter and the luciferase reporter gene (UASGx4-TK-luc).

PGPUB-DOCUMENT-NUMBER: 20020086284

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020086284 A1

TITLE: Methods for the treatment of cellular proliferative disorders

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Coffey, Matthew C.	Calgary		CA	

APPL-NO: 09/ 985756

DATE FILED: November 6, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60246728 20001109 US

US-CL-CURRENT: 435/5,435/7.23

ABSTRACT:

The present invention relates to methods of identifying the susceptibility of cells to reovirus infection by measuring constitutive ras-MAP signaling. The invention also pertains to methods using reovirus for the treatment of cellular proliferative disorders, and particularly cellular proliferative disorders wherein the proliferating cells exhibit constitutive MAPK phosphorylation, in mammals. In particular, the methods provide for reovirus treatment of mammals to treat proliferative disorders which include breast tumors, a subset of tumors in which mutation of the ras gene is not believed to play a significant role.

RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. No. 60/246,728, filed Nov. 9, 2000, the disclosure of which is hereby incorporated by reference in its entirety.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0087] For the same reasons that the treatment of breast cancers with monoclonal antibodies may be impaired (see discussion above), reovirus may be

an attractive cancer therapy. Firstly, reovirus itself is a cytocidal agent and does not rely upon immune effector cells to cause tumor regression. Indeed, the natural mechanism of killing infected cells is via direct lysis due to viral replication (Tyler and Fields, 1996). Secondly, it targets those breast cancers in which there is activation of Ras. This activation is not restricted to activating mutations of Ras (admittedly a rare subset of breast tumors) but also includes **activation of Ras caused by elements upstream** of Ras itself. These elements include not only receptor tyrosine kinases such as EGFR and HER2, but also include non-receptor tyrosine kinases such as the **Src** family members. Taken together, this type of therapy could be used with great efficacy against a tumor type as heterogeneous as breast cancer and would not be as restricted a strategy as one that targets only on receptor. Finally, the inability of antibodies to penetrate into solid tumor masses suggests that reovirus, if delivered intratumorally, should replicate unheeded. Thus, it is useful to know whether the cells of a proliferative disorder are susceptible to infection by reovirus in order to predict the efficacy of such treatment.

PGPUB-DOCUMENT-NUMBER: 20020035094

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020035094 A1

TITLE: Substituted pyridine compounds and methods of use

PUBLICATION-DATE: March 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Mantlo, Nathan B.	Lafayette	CO	US	
Schlachter, Stephen T.	Boulder	CO	US	
Josey, John A.	Longmont	CO	US	

APPL-NO: 09/ 932281

DATE FILED: August 17, 2001

RELATED-US-APPL-DATA:

child 09932281 A1 20010817 parent division-of 09642860 20000821 US PENDING child 09642860 20000821 US parent division-of 09431410 19991101 US GRANTED parent-patent 6184237 US child 09431410 19991101 US parent division-of 09185119 19981103 US GRANTED parent-patent 6022884 US non-provisional-of-provisional 60064953 19971107 US

US-CL-CURRENT: 514/151,514/336 ,514/346 ,514/352 ,546/219 ,546/268.1 ,546/308

ABSTRACT:

Selected novel substituted pyridine compounds are effective for prophylaxis and treatment of diseases, such as TNF-.alpha., IL-1.beta., IL-6 and/or IL-8 mediated diseases, and other maladies, such as pain and diabetes. The invention encompasses novel compounds, analogs, prodrugs and pharmaceutically acceptable salts thereof, pharmaceutical compositions and methods for prophylaxis and treatment of diseases and other maladies or conditions involving inflammation, pain, diabetes, cancer and the like. The subject invention also relates to processes for making such compounds as well as to intermediates useful in such processes.

--------- KWIC ---------

Summary of Invention Paragraph - BSTX:

[0014] The present invention also relates to a method of treating cancer which is mediated by Raf and Raf-inducable proteins. Raf proteins are kinases

activated in response to extracellular mitogenic stimuli such as PDGF, EGF, acidic FGF, thrombin, insulin or endothelin, and also in response to oncoproteins such as v-src, v-sis, and v-fms. Raf functions downstream of ras in signal transduction from the cellular membrane to the nucleus. Compounds in the present invention may be oncolytics through the antagonism of Raf kinase. Antisense constructs which reduce cellular levels of c-Raf and hence Raf activity inhibit the growth of rodent fibroblasts in soft agar, while exhibiting little or no general cytotoxicity. This inhibition of growth in soft agar is highly predictive of tumor responsiveness in whole animals. Moreover Raf antisense constructs have shown efficacy in reducing tumor burden in animals. Examples of cancers where Raf kinase is implicated by overexpression include cancers of the brain, larynx, lung, lymphatic system, urinary tract and stomach, including hystocytic lymphoma, lung adenocarcinoma and small cell lung cancers. Other examples include cancers involving overexpression of upstream activators of Raf or Raf-activating oncogenes, including pancreatic and breast carcinoma.

US-PAT-NO: 6458813

DOCUMENT-IDENTIFIER: US 6458813 B1

TITLE: Substituted pyridine compounds and methods of use

DATE-ISSUED: October 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mantlo; Nathan B.	Lafayette	CO	N/A	N/A
Schlachter; Stephen T.	Boulder	CO	N/A	N/A
Josey; John A.	Longmont	CO	N/A	N/A

APPL-NO: 09/ 932281

DATE FILED: August 17, 2001

PARENT-CASE:

This application is a division of application Ser. No. 09/642,860, filed Aug. 21, 2000, now U.S. Pat. No. 6,333,341, which is a division of application Ser. No. 09/431,410, filed Nov. 1, 1999, now U.S. Pat. No. 6,184,237 which is a division of application Ser. No. 09/185,119, filed Nov. 3, 1998, now U.S. Pat. No. 6,022,884 which claims the benefit of Provisional Application Serial No. 60/064,953, filed Nov. 7, 1997, which are hereby incorporated by reference.

US-CL-CURRENT: 514/335; 514/336 ; 514/340 ; 514/341 ; 514/342 ; 544/238 ; 546/261 ; 546/262 ; 546/268.1

ABSTRACT:

Selected novel substituted pyridine compounds are effective for prophylaxis and treatment of diseases, such as TNF-.alpha., IL-1.beta., IL-6 and/or IL-8 mediated diseases, and other maladies, such as pain and diabetes. The invention encompasses novel compounds, analogs, prodrugs and pharmaceutically acceptable salts thereof, pharmaceutical compositions and methods for prophylaxis and treatment of diseases and other maladies or conditions involving inflammation, pain, diabetes, cancer and the like. The subject invention also relates to processes for making such compounds as well as to intermediates useful in such processes.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX:

The present invention also relates to a method of treating cancer which is mediated by Raf and Raf-inducable proteins. Raf proteins are kinases activated in response to extracellular mitogenic stimuli such as PDGF, EGF, acidic FGF, thrombin, insulin or endothelin, and also in response to oncoproteins such as v-**src**, v-sis, and v-fms. Raf functions downstream of ras in signal transduction from the cellular membrane to the nucleus. Compounds in the present invention may be oncolytics through the antagonism of Raf kinase. Antisense constructs which reduce cellular levels of c-Raf and hence Raf activity inhibit the growth of rodent fibroblasts in soft agar, while exhibiting little or no general cytotoxicity. This inhibition of growth in soft agar is highly predictive of tumor responsiveness in whole animals. Moreover Raf antisense constructs have shown efficacy in reducing tumor burden in animals. Examples of cancers where Raf kinase is implicated by overexpression include cancers of the brain, larynx, lung, lymphatic system, urinary tract and stomach, including hystocytic lymphoma, lung adenocarcinoma and small cell lung cancers. Other examples include cancers involving overexpression of **upstream activators** of Raf or Raf-activating oncogenes, including pancreatic and breast carcinoma.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	88	hbx	USPAT; US-PGPUB	2002/10/02 12:30
2	L2	302	(hepatitis adj b adj virus or hbv) near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:31
3	L3	12	1 and 2	USPAT; US-PGPUB	2002/10/02 12:31
4	L4	5	hbx near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:35
5	L5	5066	src	USPAT; US-PGPUB	2002/10/02 12:36
6	L6	583665	activat$8	USPAT; US-PGPUB	2002/10/02 12:37
7	L7	176893	upstream	USPAT; US-PGPUB	2002/10/02 12:38
8	L8	2125	6 near5 7	USPAT; US-PGPUB	2002/10/02 12:39
9	L9	12	8 same 5	USPAT; US-PGPUB	2002/10/02 12:39
10	L10	31	5 same 6 same 7	USPAT; US-PGPUB	2002/10/02 14:35
11	L11	145	5 near2 (activator$1 or activation)	USPAT; US-PGPUB	2002/10/02 14:45
12	L12	2228	hbv or hbx	USPAT; US-PGPUB	2002/10/02 14:45
13	L13	5	11 and 12	USPAT; US-PGPUB	2002/10/02 14:45

PGPUB-DOCUMENT-NUMBER: 20020137663

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020137663 A1

TITLE: The anti-neoplastic agent ET-743 inhibits trans activation by SXR

PUBLICATION-DATE: September 26, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Forman, Barry M.	Newport Beach	CA	US	
Dussault, Isabelle	Thousand Oaks	CA	US	

APPL-NO: 09/ 927341

DATE FILED: August 13, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60224356 20000811 US

US-CL-CURRENT: 514/1

ABSTRACT:

ET-743 is a small molecular weight compound with antineoplastic activity that inhibits the ability of the nuclear receptor SXR to trans activate gene transcription from SXR regulated response elements. The nuclear receptor SXR has been identified as a receptor that activates transcription of the mdr1 gene and thus increases multidrug resistance in cells. The interaction of SXR with the mdr1 gene and ET-743 provide a set of physiological mechanisms which can be exploited to identify novel inhibitors of SXR activation and mdr1 gene transcription and thus novel agents which exhibit an antineoplastic effect against tumor cells either alone or when coadministered with another antineoplastic agent.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is related to provisional patent application Ser. No. 60/224,356, filed Aug. 11, 2000, and claims the benefit of the filing date of said provisional application.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0071] A mammalian two-hybrid assay was used to determine the effects of the Et-743 analog Pt650 on coregulator recruitment for SXR. CV-1 cells were transfected as indicated above with the indicated hybrid expression vectors and a .beta.-galactosidase vector as an internal control. Reporter activity was measured and normalized to the internal .beta.-galactosidase control and is reported as a proportion of internal .beta.-galactosidase activity. CV- 1 cells were transiently transfected with a GAL4 reporter construct and an expression vector encoding a first hybrid protein which is a DNA transcription **activator** containing the VP16 transactivation domain linked to the ligand binding domain of SXR (VP-L-SXR). In addition, cells were also transfected with expression vectors encoding the GAL4 DNA binding domain alone or a second hybrid protein which is the GAL4 DNA binding domain linked to the receptor interaction domains of the nuclear receptor coactivators **SRC** 1, ACTR, GRIP or PBP, or the nuclear receptor corepressors SMRT or NCoR, as indicated. The GAL4 reporter construct comprised four copies of a yeast GAL4 **upstream activation** sequence operatively linked to the herpes thymidine kinase promoter and the luciferase reporter gene (UASGx4-TK-luc).

PGPUB-DOCUMENT-NUMBER: 20020128280

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020128280 A1

TITLE: Methods of inducing cancer cell death and tumor regression

PUBLICATION-DATE: September 12, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Daley, George Q.	Weston	MA	US	

APPL-NO: 09/ 971545

DATE FILED: October 5, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60238240 20001005 US

US-CL-CURRENT: 514/275,514/290

ABSTRACT:

Methods are provided for treating cancer, comprising administering (1) a farnesyl protein transferase inhibitor in conjunction with (2) an additional Ras signaling pathway inhibitor to induce cancer cell death and tumor regression.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0025] Another class of signal transduction inhibitors which show an added benefit when combined with farnesyl transferase inhibitors are inhibitors of non-receptor tyrosine kinases. Like receptor tyrosine kinases, non-receptor tyrosine kinases lie **upstream** in the Ras signal transduction pathway and lead to Ras **activation**. Unlike receptor tyrosine kinases, non-receptor tyrosine kinases are not localized to the cell membrane, but are soluble proteins localized to the cytoplasm. Examples of these include the **src** and abl tyrosine kinases. In nearly all patients with chronic myelogenous leukemia, the abl tyrosine kinase is deregulated (i.e. constitutively **activated**) by a chromosomal translocation in the malignant cells leading to production of the bcr-abl fusion protein.

PGPUB-DOCUMENT-NUMBER: 20020106727

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020106727 A1

TITLE: Transcriptional intermediary factor-2

PUBLICATION-DATE: August 8, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chambon, Pierre	Blaesheim		FR	
Gronemeyer, Hinrich	Oberkirch		DE	
Voegel, Johannes	Strasbourg		FR	
Lutz, Yves	Strasbourg		FR	

APPL-NO: 09/ 842256

DATE FILED: April 26, 2001

RELATED-US-APPL-DATA:

child 09842256 A1 20010426 parent division-of 08891640 19970711 US GRANTED
parent-patent 6268173 US non-provisional-of-provisional 60021247 19960712 US

US-CL-CURRENT: 435/69.1,435/320.1 ,435/325 ,530/350 ,536/23.5

ABSTRACT:

The present invention concerns a nuclear receptor (NR) transcriptional mediator. More specifically, isolated nucleic acid molecules are provided encoding transcriptional intermediary factor-2 (TIF2). Recombinant methods for making TIF2 polypeptides are also provided as are TIF2 antibodies. Screening methods are also provided for identifying agonists and antagonists of the activation finction AF-2 of nuclear receptors, for identifying agonists and antagonists of the AD1 activation domain activity of TIF2, and for identifying agonists and antagonists of the AD2 activation domain activity of TIF2.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. application Ser. No. 60/021,247, filed Jul. 12, 1996, which is herein incorporated by reference.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0206] While the internal deletion of residues Asp.sub.1061 to Ala.sub.1070 (TIF2.19) had only a minor effect on the ability of TIF2.13 to transactivate, deletion of the Glu.sub.1071 to Leu.sub.1080 segment (mutant TIF2.20) significantly reduced TIF2 AD1 transcriptional activity. Notably, these residues belong to a sequence predicted to fold into an amphipathic .alpha.-helical structure which is highly conserved between TIF2 and **SRC**-1 (FIG. 9a). The involvement of this region in transactivation was confirmed by analysis of mutants TIF2.21 to TIF2.32 (FIGS. 9a and b). All constructs containing the TIF2 wild-type sequence from Asp.sub.1075 to Leu.sub.109 7 stimulated transcription, whereas even a deletion of only some of these residues significantly reduced transcriptional **activation**. However, on its own this .alpha.-helical peptide transactivated very poorly, and had to be incorporated into additional **upstream** and/or downstream TIF2 sequences to generate significant transcriptional activity (FIGS. 9a and b; compare mutants TIF2.13, TIF2.21 and TIF2.32). Importantly, in all cases ADD activity coincided with CBP interaction, since transcriptionally inactive constructs did not interact with CBP (TIF2.24, TIF2.27 and TIF2.29 in FIGS. 9a-c), while transcriptionally active mutants also bound CBP. Moreover, the strength of the in vitro interaction with GST-CBP apparently correlated with transactivation efficiency (FIGS. 9a-c; e.g., compare TIF2.21 and TIF2.3 1).

PGPUB-DOCUMENT-NUMBER: 20020086284

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020086284 A1

TITLE: Methods for the treatment of cellular proliferative disorders

PUBLICATION-DATE: July 4, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Coffey, Matthew C.	Calgary		CA	

APPL-NO: 09/ 985756

DATE FILED: November 6, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60246728 20001109 US

US-CL-CURRENT: 435/5,435/7.23

ABSTRACT:

The present invention relates to methods of identifying the susceptibility of cells to reovirus infection by measuring constitutive ras-MAP signaling. The invention also pertains to methods using reovirus for the treatment of cellular proliferative disorders, and particularly cellular proliferative disorders wherein the proliferating cells exhibit constitutive MAPK phosphorylation, in mammals. In particular, the methods provide for reovirus treatment of mammals to treat proliferative disorders which include breast tumors, a subset of tumors in which mutation of the ras gene is not believed to play a significant role.

RELATED APPLICATION

[0001] This application claims the benefit of U.S. Provisional Patent Application Ser. No. 60/246,728, filed Nov. 9, 2000, the disclosure of which is hereby incorporated by reference in its entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX:

[0066] Additional elements **upstream** of Ras have also been implicated in the etiology of breast cancer. The non-receptor tyrosine kinase c-**Src** has been

implicated as being as important candidate in promoting the progression of breast cancer. A number of studies indicate a -4 to -30 fold increase of c-**Src** activity in primary breast cancer tumors when compared to normal breast tissue (Verbeeks, 1996; Jacobs and Rubsamen, 1983). C-**Src** has been suggested to also play an important role in the transmission of signals from both the estrogen and progestin receptors via the Ras/MAPK pathway. A number of groups have observed that the treatment of breast tumor derived MCF-7 cells with estradiol results in the **activation** of c-**Src** kinase activity with resultant **activation** of MAPK (Di Domenico, 1996; Migliaccio, 1996). More recently, Migliaccio (1998), has demonstrated in T47D breast cancer cells that progestin stimulated cell proliferation is dependent upon Src/Ras/MAPK signaling. The fact that these steroid receptors can utilize Ras for their signaling suggests that Ras may play a more pivotal role in the promotion of growth in ER and PR positive breast tumors.

Detail Description Paragraph - DETX:

[0087] For the same reasons that the treatment of breast cancers with monoclonal antibodies may be impaired (see discussion above), reovirus may be an attractive cancer therapy. Firstly, reovirus itself is a cytocidal agent and does not rely upon immune effector cells to cause tumor regression. Indeed, the natural mechanism of killing infected cells is via direct lysis due to viral replication (Tyler and Fields, 1996). Secondly, it targets those breast cancers in which there is **activation** of Ras. This **activation** is not restricted to **activating** mutations of Ras (admittedly a rare subset of breast tumors) but also includes **activation** of Ras caused by elements **upstream** of Ras itself. These elements include not only receptor tyrosine kinases such as EGFR and HER2, but also include non-receptor tyrosine kinases such as the **Src** family members. Taken together, this type of therapy could be used with great efficacy against a tumor type as heterogeneous as breast cancer and would not be as restricted a strategy as one that targets only on receptor. Finally, the inability of antibodies to penetrate into solid tumor masses suggests that reovirus, if delivered intratumorally, should replicate unheeded. Thus, it is useful to know whether the cells of a proliferative disorder are susceptible to infection by reovirus in order to predict the efficacy of such treatment.

PGPUB-DOCUMENT-NUMBER: 20020072587

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020072587 A1

TITLE: Crystal structure of estrogen receptor-beta complex and uses thereof

PUBLICATION-DATE: June 13, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Somers, William S.	Cambridge	MA	US	
Xu, Zhang-Bao	Tewksbury	MA	US	
Akopian, Tatos N.	West Roxbury	MA	US	
Hsiao, Chu-Lai	Waltham	MA	US	
Unwalla, Rayomand	Eagleville	PA	US	

APPL-NO: 09/ 903876

DATE FILED: July 11, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60217834 20000712 US

US-CL-CURRENT: 530/350

ABSTRACT:

This invention is directed to the crystal structure of Estrogen Receptor-.beta. (ER-.beta.) complexed with genistein, and to the use of this structure in rational drug design methods to identify agents that may interact with active sites of ER-.beta., which may be useful as novel chemotherapeutic agents.

[0001] This application claims the benefit of U.S. Provisional Application Ser. No. 60/217,834 filed Jul. 12, 2000.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0053] Why does H12 occupy a different location in agonist-bound ER-.alpha. versus ER-.beta.? The only amino acid difference is Asp545 to Asn. This difference seems unlikely to explain the different positions of H12, as either residue is exposed to solvent and appears not to affect the positioning of H12. The key may lie in the residues just **upstream** of the helix which also move upon agonist/antagonist binding. Leu536 of ER-.alpha., which makes favorable

interactions in a hydrophobic cavity is replaced by Val in ER-.beta.. Val cannot make comparable contacts. Two other differences, Gly344 to Met and Asn348 to Lys, influence the nature of the H12 binding surface in this same region and may affect the position of the loop just **upstream** of H12. Changes in the position of H12 could explain the selective binding of co-**activators** to ER-.alpha. and ER-.beta.. Steroid receptor coactivator-3 (**SRC**-3) binds approximately 700 fold tighter to ER-.alpha. whereas **SRC**-1 preferentially **activates** ER-.beta. (20).

PGPUB-DOCUMENT-NUMBER: 20020048782

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020048782 A1

TITLE: PYK2 RELATED PRODUCTS AND METHODS

PUBLICATION-DATE: April 25, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
LEV, SIMA	SAN CARLOS	CA	US	
SCHLESSINGER, JOSEPH	NEW YORK	NY	US	

APPL-NO: 08/ 987689

DATE FILED: December 9, 1997

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60032824 19961211 US

US-CL-CURRENT: 435/69.1,435/325 ,435/7.1 ,530/350 ,536/23.5

ABSTRACT:

The present invention features a method for treatment of an organism having a disease or condition characterized by an abnormality in a signal transduction pathway, wherein the signal transduction pathway includes a PYK2 protein. The invention also features methods for diagnosing such diseases and for screening for agents that will be useful in treating such diseases. The invention also features purified and/or isolated nucleic acid encoding a PYK2 protein.

RELATED APPLICATIONS

[0001] The present application is related to U.S. Ser. No. 60/032,824, filed Dec. 11, 1996, entitled to PYK2 RELATED PRODUCTS AND METHODS, by Lev et al. (Lyon & Lyon Docket No. 222/126). This application is also related to U.S. application Ser. No. 08/460,626, filed Jun. 2, 1995, which is a continuation-in-part application of U.S. patent application Ser. No. 08/357,642, filed Dec. 15, 1994, both of which are incorporated herein by reference in their entirety, including any drawings.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0078] We further analyzed agonist-induced MAP kinase activity in PC12 cell lines which stably overexpress a dominant interfering mutant of Grb2 lacking the N-terminal SH3 domain (Grb2 DN-SH3) or in PC12 cells which stably overexpress the proline rich tail of Sos (Sos-CT). Xie et al., J. Biol. Chem. 270, 30717-30724 (1995); Gishizky et al., Proc. Natl. Acad. Sci. USA 92, 10889-10893 (1995). Overexpression of Grb2 DN-SH3 in PC12 cells completely blocked LPA- or bradykinin-induced MAP kinase **activation**. Overexpression of Sos-CT strongly reduced MAP kinase **activation** in response to LPA and bradykinin stimulation. However, **activation** of PYK2 or **Src** was not affected by the dominant interfering mutants of Grb2 and Sos confirming that PYK2 and **Src** act **upstream** of Grb2 and Sos in the cascade of events leading to MAP kinase **activation**.

PGPUB-DOCUMENT-NUMBER: 20020045191

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020045191 A1

TITLE: Inhibition of the SRC kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

PUBLICATION-DATE: April 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Schneider, Robert J.	New York	NY	US	
Klein, Nicola	Palo Alto	CA	US	

APPL-NO: 09/ 955006

DATE FILED: September 17, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60232892 20000915 US

US-CL-CURRENT: 435/7.1,435/15 ,514/262.1 ,514/44 ,514/520 ,514/7 ,536/24.5

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX:

[0015] The Applicants have shown that HBx **activation of Src** kinases stimulates viral DNA replication, and HBx **activates Src** kinases by stimulating two related **upstream** tyrosine kinases known as Pyk2 and p125FAK (FAK). The Applicants have

shown that HBx **activation** of Pyk2, FAK, **Src** and MAPK signalling, all occur in a calcium-dependent manner in that treatment of cells with calcium chelator (EGTA) or calcium channel poison (BAPTA-AM) specifically blocks HBx stimulation of Pyk2, which is essential for HBx activity. In addition, treatment of cells with cyclosporin A (CsA), a specific inhibitor of mitochondrial voltage-dependent anion channels, which deregulates calcium channels, also impairs HBx stimulation of HBV genomic DNA replication. Thus, the Applicants have demonstrated that HBx functions through a calcium-dependent pathway to stimulate viral DNA replication in cells and Pyk2 signal transduction, which plays a fundamental role in mammalian hepadnavirus replication.

Summary of Invention Paragraph - BSTX:

[0022] As used herein, the term "target cellular gene" refers to those genes encoding members of the **Src** kinase family, including analogs and homologs of c-**Src**, Fyn, Yes and Lyn kinases and the hematopoietic-restricted kinases HcK, Fgr, LcK and Blk, and members of the **Src** kinase signaling pathway including both **upstream** and downstream components of the **Src** signaling cascade, including members of the Pyk2-tyrosine kinase family. The proline rich tyrosine kinase, Pyk2, also known as cell adhesion kinase, CAK.beta., related adhesion focal tyrosine kinase, RAFTK, and calcium-dependent protein tyrosine kinase, (CADTK), and the closely related focal adhesion kinase (FAK) comprise a family of cytoplasmic, nonreceptor tyrosine kinases that can be regulated by extracellular stimuli and can **activate Src**-family kinases.

Detail Description Paragraph - DETX:

[0054] The present invention encompasses a variety of protocols to inhibit HBV replication and infection, including but not limited to: (1) protocols which target and inhibit HBx expression or inhibit the essential activities of HBx which may lead to **activation** of the calcium-dependent tyrosine kinase Pyk2 and the **Src** kinase signaling cascades; (2) protocols which target and inhibit **upstream** effectors of the **Src** family of kinases, such as cytosolic calcium release, which may or may not be **activated** by HBx, but are required for **activation** of Pyk2-**Src** kinase signaling cascades; and (3) protocols which target and inhibit Pyk2 tyrosine kinases, **Src** kinase family members, **Src-activated** enzymes and downstream effectors of **Src** kinases and their signal transduction pathways that are essential for viral replication.

Detail Description Paragraph - DETX:

[0061] Applicants have demonstrated that HBx **activates Src** Kinases by stimulating two related **upstream** tyrosine kinases known as Pyk2 and FAK. Both Pyk2 and FAK are **activated** by release of stored Ca.sup.2+ from controlled calcium channels. Applicants have discovered that HBx activity results in the release of stored cytosolic Ca.sup.2+ from endoplasmic reticulum and/or mitochondrial channels. While not to be limited by any mechanism of **activation**, HBx is interacting directly or indirectly with mitrochondria, to impair voltage-dependent anion channels (VDACs), a component of the

mitochondrial permeability transition pore, or Ca.sup.2+ channel, and is vital for maintaining mitochondrial Ca.sup.2+ stores, and/or endoplasmic reticulum (ER) Ca.sup.2+ pumps, known as SERCA pumps, which use ATP to maintain ER stores of Ca.sup.2+. Thus, HBx is acting directly or through other regulatory components to partially dissipate the potential of the ER SERCA pump or the mitochondrial transitional pore resulting in cytosolic calcium release.

Detail Description Paragraph - DETX:

[0088] Gene therapy approaches may also be used in accordance with the present invention to inhibit the **activation of Src** kinase and components of its signaling cascade. The gene therapy approaches described herein may also be applied to HBx, **Src** family of kinases and **upstream** and downstream effectors of the **Src** kinase signaling cascade in accordance with the present invention. Among the compounds which may disrupt the activities of HBx and its **activation of the Src** kinase signaling cascade are antisense, ribozyme, triple helix molecules, SELEX RNAs and dominant-negative mutants. Such molecules are designed to inhibit the expression of the target gene in HBV-infected host cells. Techniques for the production and use of antisense, ribozyme, triple helix and/or SELEX RNAs are well known to those of skill in the art and can be designed with respect to the cDNA sequence of **Src** kinase and components of the **Src** kinase signaling cascade.

PGPUB-DOCUMENT-NUMBER: 20020035094

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020035094 A1

TITLE: Substituted pyridine compounds and methods of use

PUBLICATION-DATE: March 21, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Mantlo, Nathan B.	Lafayette	CO	US	
Schlachter, Stephen T.	Boulder	CO	US	
Josey, John A.	Longmont	CO	US	

APPL-NO: 09/ 932281

DATE FILED: August 17, 2001

RELATED-US-APPL-DATA:

child 09932281 A1 20010817 parent division-of 09642860 20000821 US PENDING child 09642860 20000821 US parent division-of 09431410 19991101 US GRANTED parent-patent 6184237 US child 09431410 19991101 US parent division-of 09185119 19981103 US GRANTED parent-patent 6022884 US non-provisional-of-provisional 60064953 19971107 US

US-CL-CURRENT: 514/151,514/336 ,514/346 ,514/352 ,546/219 ,546/268.1 ,546/308

ABSTRACT:

Selected novel substituted pyridine compounds are effective for prophylaxis and treatment of diseases, such as TNF-.alpha., IL-1.beta., IL-6 and/or IL-8 mediated diseases, and other maladies, such as pain and diabetes. The invention encompasses novel compounds, analogs, prodrugs and pharmaceutically acceptable salts thereof, pharmaceutical compositions and methods for prophylaxis and treatment of diseases and other maladies or conditions involving inflammation, pain, diabetes, cancer and the like. The subject invention also relates to processes for making such compounds as well as to intermediates useful in such processes.

--------- KWIC ---------

Summary of Invention Paragraph - BSTX:

[0014] The present invention also relates to a method of treating cancer which is mediated by Raf and Raf-inducable proteins. Raf proteins are kinases

activated in response to extracellular mitogenic stimuli such as PDGF, EGF, acidic FGF, thrombin, insulin or endothelin, and also in response to oncoproteins such as v-**src**, v-sis, and v-fms. Raf functions downstream of ras in signal transduction from the cellular membrane to the nucleus. Compounds in the present invention may be oncolytics through the antagonism of Raf kinase. Antisense constructs which reduce cellular levels of c-Raf and hence Raf activity inhibit the growth of rodent fibroblasts in soft agar, while exhibiting little or no general cytotoxicity. This inhibition of growth in soft agar is highly predictive of tumor responsiveness in whole animals. Moreover Raf antisense constructs have shown efficacy in reducing tumor burden in animals. Examples of cancers where Raf kinase is implicated by overexpression include cancers of the brain, larynx, lung, lymphatic system, urinary tract and stomach, including hystocytic lymphoma, lung adenocarcinoma and small cell lung cancers. Other examples include cancers involving overexpression of **upstream activators** of Raf or Raf-**activating** oncogenes, including pancreatic and breast carcinoma.

US-PAT-NO: 6458813

DOCUMENT-IDENTIFIER: US 6458813 B1

TITLE: Substituted pyridine compounds and methods of use

DATE-ISSUED: October 1, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Mantlo; Nathan B.	Lafayette	CO	N/A	N/A
Schlachter; Stephen T.	Boulder	CO	N/A	N/A
Josey; John A.	Longmont	CO	N/A	N/A

APPL-NO: 09/ 932281

DATE FILED: August 17, 2001

PARENT-CASE:

This application is a division of application Ser. No. 09/642,860, filed Aug. 21, 2000, now U.S. Pat. No. 6,333,341, which is a division of application Ser. No. 09/431,410, filed Nov. 1, 1999, now U.S. Pat. No. 6,184,237 which is a division of application Ser. No. 09/185,119, filed Nov. 3, 1998, now U.S. Pat. No. 6,022,884 which claims the benefit of Provisional Application Serial No. 60/064,953, filed Nov. 7, 1997, which are hereby incorporated by reference.

US-CL-CURRENT: 514/335; 514/336 ; 514/340 ; 514/341 ; 514/342 ; 544/238 ; 546/261 ; 546/262 ; 546/268.1

ABSTRACT:

Selected novel substituted pyridine compounds are effective for prophylaxis and treatment of diseases, such as TNF-.alpha., IL-1.beta., IL-6 and/or IL-8 mediated diseases, and other maladies, such as pain and diabetes. The invention encompasses novel compounds, analogs, prodrugs and pharmaceutically acceptable salts thereof, pharmaceutical compositions and methods for prophylaxis and treatment of diseases and other maladies or conditions involving inflammation, pain, diabetes, cancer and the like. The subject invention also relates to processes for making such compounds as well as to intermediates useful in such processes.

17 Claims, 0 Drawing figures

Exemplary Claim Number: 1

---------- KWIC ---------

Brief Summary Text - BSTX:

The present invention also relates to a method of treating cancer which is mediated by Raf and Raf-inducable proteins. Raf proteins are kinases **activated** in response to extracellular mitogenic stimuli such as PDGF, EGF, acidic FGF, thrombin, insulin or endothelin, and also in response to oncoproteins such as v-**src**, v-sis, and v-fms. Raf functions downstream of ras in signal transduction from the cellular membrane to the nucleus. Compounds in the present invention may be oncolytics through the antagonism of Raf kinase. Antisense constructs which reduce cellular levels of c-Raf and hence Raf activity inhibit the growth of rodent fibroblasts in soft agar, while exhibiting little or no general cytotoxicity. This inhibition of growth in soft agar is highly predictive of tumor responsiveness in whole animals. Moreover Raf antisense constructs have shown efficacy in reducing tumor burden in animals. Examples of cancers where Raf kinase is implicated by overexpression include cancers of the brain, larynx, lung, lymphatic system, urinary tract and stomach, including hystocytic lymphoma, lung adenocarcinoma and small cell lung cancers. Other examples include cancers involving overexpression of **upstream activators** of Raf or Raf-**activating** oncogenes, including pancreatic and breast carcinoma.

US-PAT-NO: 6420338

DOCUMENT-IDENTIFIER: US 6420338 B1

TITLE: Inhibition of the Src kinase family pathway as a method of treating HBV infection and hepatocellular carcinoma

DATE-ISSUED: July 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schneider; Robert J.	New York	NY	N/A	N/A
Klein; Nicola	New York	NY	N/A	N/A

APPL-NO: 08/ 874430

DATE FILED: June 13, 1997

US-CL-CURRENT: 514/12; 514/262.1 ; 514/451 ; 514/619 ; 514/646 ; 514/789

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target HBx mediated activation of Src kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of HBV infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of HBV infection targeted to HBx and its essential activities required to sustain HBV replication. The invention is based, in part, on the Applicants' discovery that activation of Src kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that HBx mediates activation of the Src family of kinases and that this activation is a critical function provided by HBx for mammalian hepadnavirus replication.

9 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

---------- KWIC ---------

Detailed Description Text - DETX:

The present invention encompasses a variety of protocols to inhibit HBV replication and infection, including but not limited to: (1) protocols which target and inhibit HBx expression or inhibit the essential activities of HBx

which may lead to **activation of the Src** kinase signaling cascades; (2) protocols which target and inhibit **upstream** effectors of the **Src** family of kinases, which may or may not be **activated** by HBx, but are required for **activation of Src** kinase signaling cascades; and (3) protocols which target and inhibit **Src** kinase family members, **Src-activated** enzymes and downstream effectors of **Src** kinases and their signal transduction pathways that are essential for viral replication.

Detailed Description Text - DETX:

Gene therapy approaches may also be used in accordance with the present invention to inhibit the **activation of Src** kinase and components of its signaling cascade. The gene therapy approaches described herein may also be applied to HBx, **Src** family of kinases and **upstream** and downstream effectors of the **Src** kinase signaling cascade in accordance with the present invention. Among the compounds which may disrupt the activities of HBx and its **activation of the Src** kinase signaling cascade are antisense, ribozyme, triple helix molecules, SELEX RNAs and dominant-negative mutants. Such molecules are designed to inhibit the expression of the target gene in HBV-infected host cells. Techniques for the production and use of antisense, ribozyme, triple helix and/or SELEX RNAs are well known to those of skill in the art and can be designed with respect to the cDNA sequence of **Src** kinase and components of the **Src** kinase signaling cascade.

US-PAT-NO: 6406853

DOCUMENT-IDENTIFIER: US 6406853 B1

TITLE: Interventions to mimic the effects of calorie restriction

DATE-ISSUED: June 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Spindler; Stephen R.	Riverside	CA	N/A	N/A

APPL-NO: 09/ 648642

DATE FILED: August 25, 2000

PARENT-CASE:

This application is a continuation in part of U.S. application Ser. No. 09/471,225, filed Dec. 23, 1999.

US-CL-CURRENT: 435/6; 435/5 ; 435/91.1 ; 435/91.2 ; 514/693 ; 514/724

ABSTRACT:

Long term calorie restriction has the benefit of increasing life span. Methods to screen interventions that mimic the effects of calorie restriction are disclosed. Extensive analysis of genes for which expression is statistically different between control and calorie restricted animals has demonstrated that specific genes are preferentially expressed during calorie restriction. Screening for interventions which produce the same expression profile will provide interventions that increase life span. In a further aspect, it has been discovered that test animals on a calorie restricted diet for a relatively short time have a similar gene expression profile to test animals which have been on a long term calorie restricted diet.

26 Claims, 13 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 13

---------- KWIC ---------

Detailed Description Paragraph Table - DETL:

TABLE 10 mRNAs decreased by age and returned to control levels by LT-CR
GenBank Phenotype Immune System M30903 B lymphocyte kinase (Blk); **src**-family

protein tyrosine kinase; plays important role in B-cell development/activation and immune responses; B-lineage cells U43384 Cytochrome b-245, beta polypeptide (Cybb, cytochrome b558); integral component of the microbicidal oxidase electron transport chain of phagocytic cells, respiratory burst oxidase; phagocytes U10871 Mitogen **activated** protein kinase 14 (Mapk14); signal transduction, stimulate phosphorylation of transcription factors; major **upstream activator** of MAPKAP kinas 2; hematopoietic stem cells Z22649 Myeloproliferative leukemia virus oncogene (Mp1); Member of hematopoietic cytokine receptor family, cell cycle regulator, induces proliferation and differentiation of hematopoietic cell lines; hematopoietic precursor cells, platelets and megakaryocytes Y07521 Potassium voltage gated channel, Shaw-related subfamily member 1 (Kcnc1) potassium channels with properties of delayed rectifiers; nervous system, skeletal system, T lymphocytes U87456 Flavin-containing monooxygenase 1 (Fmo1); xenobiotic metabolism; highly expressed in liver, lung, kidney, lower expressed in heart, spleen, testis, brain U40189 Pancreatic polypeptide receptor 1 (Ppyr1), neuropeptide Y receptor, peptide Y receptor; G-protein-coupled receptor; liver, gastrointestinal tract, prostate, neurons endocrine cells Neuron Specific U16297 Cytochrome b-561 (Cyb561); electron transfer protein unique to neuroendocrine secretory vesicles; vectoral transmembrane electron transport; brain D50032 Trans-golgi network protein 2 (Ttgn2); integral membrane protein localized to the trans-Golgi network; involved in the budding of exocytic transport vesicles; brain neurons Liver Specific/Ubiquitous D82019 Basigin (Bsg), CD147, neurothelin; membrane glycoprotein, immunoglobulin superfamily, homology to MHCs, acts as an adhesion molecule or a receptor, neural network formation and tumor progression; embryo, liver and other organs L38990 Glucokinase (Gk), key glycolytic enzyme; liver U50631 Heat-responsive protein 12 (Hrsp12); heat-responsive, phosphorylated protein sequence simularity to Hsp70; liver, kidney U39818 Tuberous sclerosis 2 (Tsc2); mutationally inactivated in some families with tuberous sclerosis; encodes a large, membrane-associated GTPase **activating** protein (GAP tuberlin); may have a key role in the regulation of cellular growth; ubiquitous

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	88	hbx	USPAT; US-PGPUB	2002/10/02 12:30
2	L2	302	(hepatitis adj b adj virus or hbv) near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:31
3	L3	12	1 and 2	USPAT; US-PGPUB	2002/10/02 12:31
4	L4	5	hbx near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:35
5	L5	5066	src	USPAT; US-PGPUB	2002/10/02 12:36
6	L6	583665	activat$8	USPAT; US-PGPUB	2002/10/02 12:37
7	L7	176893	upstream	USPAT; US-PGPUB	2002/10/02 12:38
8	L8	2125	6 near5 7	USPAT; US-PGPUB	2002/10/02 12:39
9	L9	12	8 same 5	USPAT; US-PGPUB	2002/10/02 12:39
10	L10	31	5 same 6 same 7	USPAT; US-PGPUB	2002/10/02 14:35
11	L11	145	5 near2 (activator$1 or activation)	USPAT; US-PGPUB	2002/10/02 14:45
12	L12	2228	hbv or hbx	USPAT; US-PGPUB	2002/10/02 14:45
13	L13	5	11 and 12	USPAT; US-PGPUB	2002/10/02 14:45

PGPUB-DOCUMENT-NUMBER: 20020045191

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020045191 A1

TITLE: Inhibition of the SRC kinase family pathway as a method of treating **HBV** infection and hepatocellular carcinoma

PUBLICATION-DATE: April 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Schneider, Robert J.	New York	NY	US	
Klein, Nicola	Palo Alto	CA	US	

US-CL-CURRENT: 435/7.1;435/15 ;514/262.1 ;514/44 ;514/520 ;514/7 ;536/24.5

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target **HBx** mediated **activation of Src** kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of **HBV** infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of **HBV** infection targeted to **HBx** and its essential activities required to sustain **HBV** replication. The invention is based, in part, on the Applicants' discovery that **activation of Src** kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that **HBx** mediates **activation of the Src** family of kinases and that this activation is a critical function provided by **HBx** for mammalian hepadnavirus replication.

US-PAT-NO: 6420338

DOCUMENT-IDENTIFIER: US 6420338 B1

TITLE: Inhibition of the Src kinase family pathway as a method of treating **HBV** infection and hepatocellular carcinoma

DATE-ISSUED: July 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schneider; Robert J.	New York	NY	N/A	N/A
Klein; Nicola	New York	NY	N/A	N/A

US-CL-CURRENT: 514/12; 514/262.1 ; 514/451 ; 514/619 ; 514/646 ; 514/789

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target **HBx** mediated **activation of Src** kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of **HBV** infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of **HBV** infection targeted to **HBx** and its essential activities required to sustain **HBV** replication. The invention is based, in part, on the Applicants' discovery that **activation of Src** kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that **HBx** mediates **activation of the Src** family of kinases and that this activation is a critical function provided by **HBx** for mammalian hepadnavirus replication.

9 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

US-PAT-NO: 6365372

DOCUMENT-IDENTIFIER: US 6365372 B1

TITLE: SNF2 related CBP activator protein (SRCAP)

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chrivia; John	Kirkwood	MO	N/A	N/A
Yaciuk; Peter	Webster Groves	MO	N/A	N/A

APPL-NO: 09/ 579181

DATE FILED: May 25, 2000

PARENT-CASE:

This application claims the benefit of U.S. Provisional Application No. 60/136,620, filed May 27, 1999, incorporated in its entirety herein by reference.

US-CL-CURRENT: 435/69.1; 435/16 ; 530/350 ; 530/389.1 ; 536/23.1

ABSTRACT:

A protein, SRCAP, is provided. The protein is capable of co-activating CREB binding protein (CBP) mediated transcription, as well as activating transcription without CBP. SRCAP is a Snf2 family member. As such, it has ATPase activity. Fragments of SRCAP are also provided, as are polynucleotides encoding SRCAP and its fragments. Antibodies that bind to SRCAP are also provided. These compositions are useful for enhancing transcription in cells and patients. The compositions are also useful for reducing transcription in cells and patients.

30 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

---------- KWIC ---------

Brief Summary Text - BSTX:

CBP is a histone acetyltransferase capable of acetylating not only histones but also several transcription factors such as GATA-1 and p53 (Boyes et al., 1998,

Nature 396, 594-598; Hung et al., 1999, Cell Biol. 19, 3496-3505; Webster et al., 1999, Mol. Cell. Biol. 19, 3485-3495). CBP also binds to several proteins that also finction as histone acetyltransferases (P/CAF, p/CIP and the p160 co-**activators such as SRC**-1).

Brief Summary Text - BSTX:

CBP is critical for the functioning of several viruses that impact human health. This includes human T cell lymphotrophic virus (HTLV-1), which recruits CBP to viral promoters through interaction of the viral protein TAX with CREB (Kwok et at., 1996). CBP also interacts directly with the viral transactivator protein Tat of human immunodeficiency virus type I (mHV-1) (Hottiger and Nabel, 1998, J. Virol. 72, 8252-8256). In humans, adenoviruses cause several diseases including: acute follicular conjunctivitis, pharyngoconjunctivial fever, epidermic keratoconjunctivitis, acute hemorrhagic cystitis, cervicitis, infantile diarrhea, and respiratory tract infections in children. Immune compromised individuals such as those who have undergone bone or organ transplant or who have AIDS are particularly susceptible to adenoviral caused diseases (reviewed in Chapter 65 of Zinsser, 1992, Microbiology, 20.sup.th Ed., Joklik et al., Eds., Appleton and Lange). CBP interacts with several isoforms of the adenoviral protein EIA to mediate repression or activation of transcription (Felzien et al., 1999, Mol. Cell. Biol. 19,42616). CBP also functions as a co-activator for the zta protein of Epstein-Barr virus (Zerby et al., 1999, Moll. Cell. Biol. 19, 1617-1626). Epstein-Barr virus infections have been associated with fatal lymphoproliferation in immune deficient patients and the development of Burkitt's lymphoma (reviewed in Zinsser Microbiology, 1992, supra). CBP has also been indirectly implicated in the transcriptional regulation of other viruses through interaction with CREB. For example, CREB binds to the enhancer of the IE1/2 gene of human cytomegalovirus (Lang et al., 1992, Nucl. Acids Res. 20, 3287-3295), an important pathogen in immunosuppressed patients such as transplant recipients and AIDS patients (Drew, 1988, J. Infect. Dis. 158, 449456). CREB also binds the hepatitis B virus (**HBV**) enhancer element when complexed with the **HBV** protein, pX (Maguire et al., 1991, Science 252, 842-844). A possible role of CBP in some forms of cancer is suggested by the observation that CBP is part of a multi-subunit complex with the breast cancer tumor suppressor BRCA1 and RNA polymerase II (Neish et al., 1998, Nucl. Acids Res. 26, 847-853). In some forms of acute myeloid leukemia a t(7;11)(p15: p15) translocation results in the NUP98-HOXA9 fusion protein, which is a strong transcriptional activator that uses CBP as a co-activator (Kasper et al., 1999).

Detailed Description Text - DETX:

SRCAP would also be expected to affect several aspects of virus infection. Examples include the interaction of the HTILV-1 protein TAX with CREB; the interaction of CBP with the HIV-1 Tat protein; various effects of adenovirus infection due to the interaction of CBP with the adenovirus E1A protein; various effects of Epstein-Barr virus due to the interaction of CBP with the viral zta protein; various effects of cytomegalovirus due to the interaction of CREB with the enhancer of the viral IE1/2 gene; various effects of hepatitis B

virus (**HBV**) due to the interaction of CREB with the viral protein pX; and various effects of hepatitis C virus due to the interaction of SRCAP with the viral protein NS5A (see Example 2).

Detailed Description Text - DETX:

The present invention also provides methods of treating a patient having a disease involving a function affected by SRCAP. The methods comprise treating a cell of the patient with an amount of a polypeptide, polynucleotide, or antibody composition of the invention sufficient to modulate transcription of the cell. As described supra in the context of the cell treatment methods, the treatment can be directed toward increasing the presence of SRCAP (e.g., by SRCAP or SRCAP fragment polypeptide or polynucleotide) or decreasing the presence of SRCAP (e.g., by antisense, ribozyme or antibody treatment). In particular embodiments, the function is insufficient transcription of a gene selected from the group consisting of a gene mediated by CBP co-activation, a DEAD box RNA dependent helicase (see Example 3), adenoviral DBP protein, .beta.-actin, and a nuclear receptor. An example of a nuclear receptor is the glucocorticoid receptor (GR). In these embodiments, the cell is treated with a SRCAP polypeptide or SRCAP polynucleotide, or fragments thereof. In other embodiments, the function is undesired effects caused by HCV NS5A, HTLV-1 TAX protein, HIV-1 Tat protein, adenovirus EIA protein, Epstein-Barr virus zta protein, cytomegalovirus IE1/2 enhancer, hepatitis B virus (**HBV**) pX protein. As previously discussed, when reduction of transcription is desired, the cell may be treated with SRCAP antisense or ribozyme preparations, or with anti-SRCAP antibodies.

	L #	Hits	Search Text	DBs	Time Stamp
1	L1	88	hbx	USPAT; US-PGPUB	2002/10/02 12:30
2	L2	302	(hepatitis adj b adj virus or hbv) near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:31
3	L3	12	1 and 2	USPAT; US-PGPUB	2002/10/02 12:31
4	L4	5	hbx near4 inhibit$8	USPAT; US-PGPUB	2002/10/02 12:35
5	L5	5066	src	USPAT; US-PGPUB	2002/10/02 12:36
6	L6	583665	activat$8	USPAT; US-PGPUB	2002/10/02 12:37
7	L7	176893	upstream	USPAT; US-PGPUB	2002/10/02 12:38
8	L8	2125	6 near5 7	USPAT; US-PGPUB	2002/10/02 12:39
9	L9	12	8 same 5	USPAT; US-PGPUB	2002/10/02 12:39
10	L10	31	5 same 6 same 7	USPAT; US-PGPUB	2002/10/02 14:35
11	L11	145	5 near2 (activator$1 or activation)	USPAT; US-PGPUB	2002/10/02 14:45
12	L12	2228	hbv or hbx	USPAT; US-PGPUB	2002/10/02 14:45
13	L13	5	11 and 12	USPAT; US-PGPUB	2002/10/02 14:45
14	L14	365	5 near5 6	USPAT; US-PGPUB	2002/10/02 14:54
15	L15	5	12 and 13	USPAT; US-PGPUB	2002/10/02 14:56
16	L16	15	12 and 14	USPAT; US-PGPUB	2002/10/02 14:56

PGPUB-DOCUMENT-NUMBER: 20020115215

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020115215 A1

TITLE: Targeted modification of chromatin structure

PUBLICATION-DATE: August 22, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wolffe, Alan P.	Orinda	CA	US	
Collingwood, Trevor	San Pablo	CA	US	

APPL-NO: 09/ 844508

DATE FILED: April 27, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60200590 20000428 US
non-provisional-of-provisional 60228523 20000828 US

US-CL-CURRENT: 435/455,435/468 ,435/6

ABSTRACT:

Methods and compositions for targeted modification of chromatin structure, within a region of interest in cellular chromatin, are provided. Such methods and compositions are useful for facilitating processes such as, for example, transcription and recombination, that require access of exogenous molecules to chromosomal DNA sequences.

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority under the provisions of 35 U.S.C. .sctn.119 to U.S. Provisional Patent Application Serial No. 60/200,590, filed Apr. 28, 2000 and U.S. Provisional Patent Application Serial No. 60/228,523, filed Aug. 28, 2000; the disclosures of which are hereby incorporated by reference in their entireties.

---------- KWIC ---------

Detail Description Paragraph - DETX:

[0122] Numerous HAT enzymes have been described, including budding yeast Gen5p, which is required for expression of a subset of the yeast genome, its mammalian

orthologue CREB-binding protein (CBP), p300 (both of the latter two used as coactivators by a wide variety of mammalian transcription factors), TAF.sub.II250 (a component of the basal transcriptional machinery), and steroid receptor coactivator 1 (**SRC-1), which potentiates transcriptional activation** by a number of nuclear hormone receptors. Kouzarides (1999) supra; Cheung et al. (2000) Curr. Opin. Cell Biol. 12:326-333; and Sterner et al. (2000) supra.

Detail Description Paragraph - DETX:

[0196] Expression of human, mammalian, bacterial, fungal, protozoal, Archaeal, plant and viral genes can be modulated; viral genes include, but are not limited to, hepatitis virus genes such as, for example, **HBV**-C, **HBV**-S, **HBV**-X and **HBV**-P; and HIV genes such as, for example, tat and rev. Modulation of expression of genes encoding antigens of a pathogenic organism can be achieved using the disclosed methods and compositions.

Detail Description Paragraph - DETX:

Activation of EPO Expression by Fusion of SRC-1 to a Zinc Finger Binding Domain

PGPUB-DOCUMENT-NUMBER: 20020045191

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020045191 A1

TITLE: Inhibition of the SRC kinase family pathway as a method of treating **HBV** infection and hepatocellular carcinoma

PUBLICATION-DATE: April 18, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Schneider, Robert J.	New York	NY	US	
Klein, Nicola	Palo Alto	CA	US	

US-CL-CURRENT: 435/7.1;435/15 ;514/262.1 ;514/44 ;514/520 ;514/7 ;536/24.5

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target **HBx** mediated **activation of Src** kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of **HBV** infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of **HBV** infection targeted to **HBx** and its essential activities required to sustain **HBV** replication. The invention is based, in part, on the Applicants' discovery that **activation of Src** kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that **HBx** mediates **activation of the Src family of kinases and that this activation** is a critical function provided by **HBx** for mammalian hepadnavirus replication.

PGPUB-DOCUMENT-NUMBER: 20020031522

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20020031522 A1

TITLE: TRUNCATED CRAFI INHIBITS CD40 SIGNALING

PUBLICATION-DATE: March 14, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
BALTIMORE, DAVID	BOSTON	MA	US	
CHENG, GENHONG	LOS ANGELES	CA	US	
YE, ZHENG-SHENG	NEW YORK	NY	US	
LEDERMAN, SETH	NEW YORK	NY	US	
CLEARY, AILEEN	NEW YORK	NY	US	

APPL-NO: 08/ 813323

DATE FILED: March 10, 1997

CONTINUED PROSECUTION APPLICATION: This is a publication of a continued prosecution application (CPA) filed under 37 CFR 1.53(d).

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60013199 19960311 US

US-CL-CURRENT: 424/185.1,435/63 ,435/64 ,435/7.2 ,435/7.23 ,530/324 ,530/350 ,530/358

ABSTRACT:

Overexpression of a CRAF1 (CD40 receptor-associated factor 1) gene truncated by 323 to about 414 amino acids at the amino inhibits CD40-mediated cell activation, and is used to treat conditions characterized by an unwanted level of CD40-mediated intracellular signaling.

[0001] This application claims the benefit of U.S. Provisional No. 60/013,199, filed Mar. 11, 1996, the contents of which are hereby incorporated by reference into the present application.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX:

[0006] Stimulation of CD40 causes the tyrosine phosphorylation of multiple

substrates including **Src family kinases such as p53-p56.sup.lyn, activates** multiple serine-threonine-specific protein kinases, and induces the phosphorylation of phospholipase C-.gamma.2 and of phosphoinositide-3' kinase (14).

Detail Description Paragraph - DETX:

[0073] Specific viral vectors for use in gene transfer systems are now well established. See for example: Madzak et al., J. Gen. Virol., 73: 1533-36 (1992: papovavirus SV40); Berkner et al., Curr. Top. Microbiol. Immunol., 158: 39-61 (1992: adenovirus); Moss et al., Curr. Top. Microbiol. Immunol., 158: 25-38 (1992: vaccinia virus); Muzyczka, Curr. Top. Microbiol. Immunol., 158: 97-123 (1992: adeno-associated virus); Margulskee, Curr. Top. Microbiol. Immunol., 158: 67-93 (1992: herpes simplex virus (HSV) and Epstein-Barr virus (**HBV**)); Miller, Curr. Top. Microbiol. Immunol., 158: 1-24 (1992:retrovirus); Brandyopadhyay et al., Mol. Cell. Biol., 4: 749-754 (1984: retrovirus); Miller et al., Nature, 357: 455-450 (1992: retrovirus); Anderson, Science, 256: 808-813 (1992:retrovirus), all of which are incorporated herein by reference.

US-PAT-NO: 6420338

DOCUMENT-IDENTIFIER: US 6420338 B1

TITLE: Inhibition of the Src kinase family pathway as a method of treating **HBV** infection and hepatocellular carcinoma

DATE-ISSUED: July 16, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Schneider; Robert J.	New York	NY	N/A	N/A
Klein; Nicola	New York	NY	N/A	N/A

US-CL-CURRENT: 514/12; 514/262.1 ; 514/451 ; 514/619 ; 514/646 ; 514/789

ABSTRACT:

The present invention relates to therapeutic protocols and pharmaceutical compositions designed to target **HBx** mediated **activation of Src** kinase, members of the Src tyrosine kinase family and components of the Src kinase family signal transduction pathways for the treatment of **HBV** infection and related disorders and diseases, such as HCC. The invention further relates to pharmaceutical compositions for the treatment of **HBV** infection targeted to **HBx** and its essential activities required to sustain **HBV** replication. The invention is based, in part, on the Applicants' discovery that **activation of Src** kinase signaling cascades play a fundamental role in mammalian hepadnavirus replication. Applicants have demonstrated that **HBx** mediates **activation of the Src family of kinases and that this activation** is a critical function provided by **HBx** for mammalian hepadnavirus replication.

9 Claims, 14 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 8

US-PAT-NO: 6376236

DOCUMENT-IDENTIFIER: US 6376236 B1

TITLE: Recombinant alphavirus particles

DATE-ISSUED: April 23, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dubensky, Jr.; Thomas W.	Rancho Sante Fe	CA	N/A	N/A
	San Diego	CA	N/A	N/A
Polo; John M.	San Diego	CA	N/A	N/A
Ibanez; Carlos E.	San Diego	CA	N/A	N/A
Driver; David A.				

APPL-NO: 09/ 236140

DATE FILED: January 22, 1999

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS The present application is a divisional of U.S. patent application Ser. No. 08/404,796, filed Mar. 15, 1995 U.S. Pat. No. 6,015,686, which application is a continuation-in-part of U.S. patent application Ser. No. 08/376,184, filed Jan. 18, 1995 now abandoned, which application is a continuation-in-part of U.S. patent application Ser. No. 08/348,472, filed Nov. 30, 1994, now abandoned, which application is a continuation-in-part of U.S. patent application Ser. No. 08/198,450, filed Feb. 18, 1994, now abandoned, which application is a continuation-in-part of U.S. patent application Ser. No. 08/122,791, filed Sep. 15, 1993, now abandoned.

US-CL-CURRENT: 435/320.1

ABSTRACT:

Disclosed are recombinant alphavirus particles comprising a) an alphavirus vector construct which directs the expression of a heterologous nucleic acid molecule; b) a capsid protein; and c) an envelope glycoprotein from a virus different from said alphavirus vector.

11 Claims, 37 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 30

---------- KWIC ---------

Brief Summary Text - BSTX:

In still other embodiments, the vectors described above include a selected *heterologous sequence which may be obtained from a virus selected from the group consisting of influenza virus, HPV, **HBV,** HCV, EBV, HIV, HSV, FeLV, FIV, Hanta virus, HTLV I, HTLV II and CMV. Within one preferred embodiment, the heterologous sequence obtained from HPV encodes a protein selected from the group consisting of E5, E6, E7 and L1. In yet other embodiments, the vectors described above include a selected heterologous sequence encoding an HIV protein selected from the group consisting of HIV gp120 and gag.

Brief Summary Text - BSTX:

The selected heterologous sequences described above also may be an antisense sequence, noncoding sense sequence, or ribozyme sequence. In preferred embodiments, the antisense or noncoding sense sequence is selected from the group consisting of sequences which are complementary to influenza virus, HPV, **HBV,** HCV, EBV, HIV, HSV, FeLV, FIV, Hanta virus, HTLV I, HTLV II, and CMV sequences.

Brief Summary Text - BSTX:

Within another aspect, **HBV**-derived and coronavirus-derived packaging cell lines are provided which are suitable for packaging and production of and alphavirus vector. Within one embodiment, an **HBV**-derived packaging cell line is provided, comprising an expression cassette which directs the expression of **HBV** core, preS/S, and P proteins. Within another embodiment, a coronavirus-derived packaging cell line is provided, comprising an expression cassette which directs the expression of coronavirus N, M, and S proteins.

Detailed Description Text - DETX:

Within related aspects of the present invention, alphavirus cell-specific expression vectors may be constructed to express viral antigens, ribozyme, antisense sequences or immunostimulatory factors such as gamma-interferon (.gamma.-IFN), IL-2 or IL-5 for the targeted treatment of virus infected cell types. In particular, in order to target alphavirus vectors to specific foreign organism or pathogen-infected cells, inverted repeats of the alphavirus vector may be selected to hybridize to any pathogen-specific RNA, for instance target cells infected by pathogens such as HIV, CMV, **HBV,** HPV and HSV.

Detailed Description Text - DETX:

Another example of an immunomodulatory cofactor is the B7/BB1 costimulatory factor. Briefly, activation of the full functional activity of T cells requires two signals. One signal is provided by interaction of the antigen-specific T cell receptor with peptides which are bound to major

histocompatibility complex (MHC) molecules, and the second signal, referred to as costimulation, is delivered to the T cell by antigen-presenting cells. Briefly, the second signal is required for interleukin-2 (IL-2) production by T cells and appears to involve interaction of the B7/BB1 molecule on antigen-presenting cells with CD28 and CTLA-4 receptors on T lymphocytes (Linsley et al., J. Exp. Med., 173:721-730, 1991a, and J. Exp. Med., 174:561-570, 1991). Within one embodiment of the invention, B7/BB1 may be introduced into tumor cells in order to cause costimulation of CD8.sup.+ T cells, such that the CD8.sup.+ T cells produce enough IL-2 to expand and become fully activated. These CD8.sup.+ T cells can kill tumor cells that are not expressing B7 because costimulation is no longer required for further CTL function. Vectors that express both the costimulatory B7/BB1 factor and, for example, an immunogenic **HBV** core protein, may be made utilizing methods which are described herein. Cells transduced with these vectors will become more effective antigen-presenting cells. The **HBV** core-specific CTIL response will be augmented from the fully activated CD8.sup.+ T cell via the costimulatory ligand B7/BB1.

Detailed Description Text - DETX:

Within other aspects of the present invention, alphavirus vector constructs are provided which direct the expression of immunogenic portions of antigens from foreign organisms or other pathogens. Representative examples of such antigens include bacterial antigens (e.g., E. coli, streptococcal, staphylococcal, mycobacterial, etc.), fungal antigens, parasitic antigens, and viral antigens (e.g., influenza virus, Human Immmunodeficiency Virus ("HIV"), Hepatitis A, B and C Virus ("HAV", "**HBV**" and "HCV", respectively), Human Papiloma Virus ("HPV"), Epstein-Barr Virus ("EBV"), Herpes Simplex Virus ("HSV"), Hantavirus, TTLV I, HTLV II and Cytomegalovirus ("CMV"). As utilized within the context of the present invention, "immunogenic portion" refers to a portion of the respective antigen which is capable, under the appropriate conditions, of causing an immune response (i.e., cell-mediated or humoral). "Portions" may be of variable size, but are preferably at least 9 amino acids long, and may include the entire antigen. Cell-mediated immune responses may be mediated through Major Histocompatability Complex ("MHC") class I presentation, MHC Class II presentation, or both.

Detailed Description Text - DETX:

Within one aspect of the invention, alphavirus vector constructs are provided which direct the expression of immunogenic portions of Hepatitis B antigens. Briefly, the Hepatitis B genome is comprised of circular DNA of about 3.2 kilobases in length and has been well characterized (Tiollais et al., Science 213:406-411, 1981; Tiollais et al., Nature 317:489-495, 1985; and Ganem and Varmus, Ann. Rev. Biochem. 56:651-693, 1987; see also EP 0 278,940, EP 0 241,021, WO 88/10301, and U.S. Pat. Nos. 4,696,898 and 5,024,938, which are hereby incorporated by reference). The Hepatitis B virus presents several different antigens, including among others, three HB "S" antigens (HBsAgs), an HBc antigen (HBcAg), an HBe antigen (HBeAg), and an **HBx** antigen (HBxAg) (see Blum et al., TIG 5(5):154158, 1989). Briefly, the HBeAg results from proteolytic cleavage of a P22 pre-core intermediate and is secreted from the

cell. HBeAg is found in serum as a 17 kD protein. The HBcAg is a protein of 183 amino acids, and the HBxAg is a protein of 145 to 154 amino acids, depending on subtype.

Detailed Description Text - DETX:

As will be evident to one of ordinary skill in the art, various immunogenic portions of the above-described S antigens may be combined in order to induce an immune response when administered by one of the alphavirus vector constructs described herein. In addition, due to the large immunological variability that is found in different geographic regions for the S open reading frame of **HBV**, particular combinations of antigens may be preferred for administration in particular geographic regions. Briefly, epitopes that are found in all human hepatitis B virus S samples are defined as determinant "a". Mutually exclusive subtype determinants, however, have also been identified by two-dimensional double immunodiffusion (Ouchterlony, Progr. Allergy 5:1, 1958). These determinants have been designated "d" or "y" and "w" or "r" (LeBouvier, J. Infect. 123:671, 1971; Bancroft et al., J. Immunol. 109:842, 1972; and Courouce et al., Bibl. Haematol. 42:1-158, 1976). The immunological variability is due to single nucleotide substitutions in two areas of the hepatitis B virus S open reading frame, resulting in the following amino acid changes: (1) exchange of lysine-122 to arginine in the Hepatitis B virus S open reading frame causes a subtype shift from d to y, and (2) exchange of arginine-160 to lysine causes the shift from subtype r to w. In Africans, subtype ayw is predominant, whereas in the U.S. and northern Europe the subtype adw.sub.2 is more abundant (Molecular Biology of the Hepatitis B Virus, McLachlan (ed.), CRC Press, 1991). As will be evident to one of ordinary skill in the art, it is generally preferred to construct a vector for administration which is appropriate to the particular hepatitis B virus subtype which is prevalent in the geographical region of administration. Subtypes of a particular region may be determined by two-dimensional double immunodiffusion or, preferably, by sequencing the S open reading frame of **HBV** virus isolated from individuals within that region.

Detailed Description Text - DETX:

Also presented by **HBV** are pol ("**HBV** pol"), ORF 5, and ORF 6 antigens. Briefly, the polymerase open reading frame of **HBV** encodes reverse transcriptase activity found in virions and core-like particles in infected livers. The polymerase protein consists of at least two domains: the amino terminal domain which encodes the protein that primes reverse transcription, and the carboxyl terminal domain which encodes reverse transcriptase and RNase H activity. Immunogenic portions of **HBV** pol may be determined utilizing methods described herein (e.g., below and in Example 13), utilizing alphavirus vector constructs described below, and administered in order to generate an immune response within a warm-blooded animal. Similarly, other **HBV** antigens, such as ORF 5 and ORF 6 (Miller et al., Hepatology 9:322-327, 1989) may be expressed utilizing alphavirus vector constructs as described herein. Representative examples of alphavirus vector constructs utilizing ORF 5 and ORF 6 are set forth below in the examples.

Detailed Description Text - DETX:

A wide variety of vector systems may be utilized as the first layer of the eukaryotic layered vector initiation system, including for example, viral vector constructs developed from DNA viruses such as those classified in the Poxviridae, including for example canary pox virus or vaccinia virus (e.g., Fisher-Hoch et al., PNAS 86:317-321, 1989; Flexner et al., Ann. N.Y. Acad. Sci. 569:86-103, 1989; Flexner et al., Vaccine 8:17-21, 1990; U.S. Pat. Nos. 4,603,112, 4,769,330 and 5,017,487; WO 89/01973); Papoviridae such as BKV, JCV or SV40 (e.g., Mulligan et al., Nature 277:108-114, 1979); Adenoviridae, such as adenovirus (e.g., Berkner, Biotechniques 6:616-627, 1988; Rosenfeld et al., Science 252:431-434, 1991); Parvoviridae, such as adeno-associated virus (e.g., Samulski et al., J. Vir. 63:3822-3828, 1989; Mendelson et al., Virol. 166:154-165, 1988; PA 7/222,684); Herpesviridae, such as Herpes Simplex Virus (e.g., Kit, Adv. Exp. Med. Biol. 215:219-236, 1989); and Hepadnaviridae (e.g., **HBV**), as well as certain RNA viruses which replicate through a DNA intermediate, such as the Retroviridae (see, e.g., U.S. Pat. No. 4,777,127, GB 2,200,651, EP 0,345,242 and WO91/02805; Retroviridae include leukemia in viruses such as MoMLV and immunodeficiency viruses such as HIV, e.g., Poznansky, J. Virol. 65:532-536, 1991).

Detailed Description Text - DETX:

Within other aspects of the present invention, compositions and methods are provided for administering an alphavirus vector construct which is capable of preventing, inhibiting, stabilizing or reversing infectious, cancerous, auto-immune or immune diseases. Representative examples of such diseases include viral infections such as HIV, **HBV** HTLV I, HLTV II, CMV, EBV and HPV, melanomas, diabetes, graft vs. host disease, Alzheimer's disease and heart disease.

Detailed Description Text - DETX:

Using techniques described above, the lacZ gene encoding the .beta.-galactosidase reporter protein was cut from the plasmid pSV-.beta.-galactosidase (PROMEGA CORP, Madison, Wis.) and substituted into the ELVIS-luc plasmid DNA vector in place of luciferase. To examine in vivo gene expression from ELVIS vectors, Balb/c mice and rats are injected intramuscularly (i.m.) with ELVIS-.beta.-gal or ELVIS-luc plasmid DNA vectors. FIG. 24 demonstrates the in vivo expression of .beta.-galactocidase in muscle tissue taken from a rat and stained with X-gal at three days post i.m. injection. Mice injected with ELVIS-.beta.-gal also demonstrate positively staining blue muscle fibers. Luciferase expression levels from muscle which were between 75- and 300-fold higher than control levels were detected in 3/4 Balb/c mice at two days post i.m. inoculation with ELVIS-luc plasmid. In other experiments, C3H/HeN mice were injected i.m. with ELVIS vectors expressing either the hepatitis B virus core (**HBV**-core) or hepatitis B virus e (**HBV**-e) proteins. Using ELISA detection systems, both **HBV**-core- and **HBV**-e-specific IgG antibodies were detected in serum samples collected from the mice 10 days following the second injection with the vectors. These

experiments demonstrate that Sindbis-derived DNA vectors are able to express foreign genes in vivo, in rat and mouse muscle.

Detailed Description Text - DETX:

In the derivation of alphavirus vector packaging and producer cell lines, many approaches are outlined to control the expression of viral genes, such that producer cell lines stably transformed with both vector and vector packaging cassettes, can be derived. These approaches include inducible and/or cellular differentiation sensitive promoters, antisense structural genes, heterologous control systems, and mosquito or other cells in which viral persistent infections are established. Regardless of the final configuration for the alphavirus vector producer cell line, the ability to establish persistent infection, or at least delay cell death as a result of viral gene expression, may be enhanced by inhibiting apoptosis. For example, the DNA tumor viruses, including adenovirus, HPV, SV40, and mouse polyomavirus (Py), transform cells in part, by binding to, and inactivating, the retinoblastoma (Rb) gene product p105 and its closely related gene product, p107, and other gene products involved in the control of the cell cycle including cyclin A, p33.sup.cdk2 and p34.sup.cdc2. All of these viruses, except for Py, encode gene products which bind to and inactivate p53. Uniquely, Py encodes middle T antigen (mT) which binds to and **activates the membrane tyrosine kinase, src,** and also phosphatidylinositol-3-kinase, which is required for the full transformation potential of this virus (Talmage et al., Cell 59:55-65, 1989). The binding to and inactivation of the Rb and p53 recessive oncogene products prevents cells transformed by these DNA tumor viruses from entering the apoptotic pathway. It is known that p53 is able to halt the division of cells, in part by inhibiting the expression of proteins associated with cellular proliferation, including c-fos, hsc70, and bcl-2 (Miyashita et al., Cancer Research 54:3131-3135,1994).

Detailed Description Text - DETX:

In another embodiment, a RNA packaging signal derived from another virus is inserted into the alphavirus vector to allow packaging by the structural proteins of that corresponding virus. For example, the 137 nt. packaging signal from hepatitis B virus, located between nts. 3134 and 88 and spanning the precore/core junction (Junker-Niepmann et al. EMBO J. 9:3389, 1990), is amplified from an **HBV** template using two oligonucleotide primers. PCR is performed using a standard three temperature cycling protocol, plasmid pHBV1.1 (Junker-Niepmann et al. EMBO J. 9:3389, 1990) as the template, and the following oligonucleotide pair, each of which contain 20 nucleotides complementary to the **HBV** sequence and flanking ApaI recognition sequences:

Detailed Description Text - DETX:

Following amplification, the PCR amplicon is digested with ApaI and purified from a 1.5% agarose gel using MERMAID.TM. (Bio101). Sindbis vector plasmid pKSSINdIJRsjrc (Example 3) also is digested with ApaI, under limited conditions to cleave at only one of its two sites, followed by treatment with CIAP, purification from a 1% agarose gel, and ligation with the above-synthesized **HBV**

amplicon, to produce a construct designated pKSSINhbvJR. Other alphavirus vectors (see Example 3) are readily modified in a similar manner. Cell lines which express the **HBV** core, preS/S, and P proteins necessary for packaging of the RNA sequence are derived by modification of helper plasmid pCH3143 (Junker-Niepmann et al., EMBO J. 9:3389, 1990) to include a selectable marker. An expression cassette containing the neomycin resistance marker is obtained by digestion of plasmid pBK-RSV (Stratagene) with Mst II and blunt-ending with Klenow fragment. The selectable marker is then ligated into any one of several unique sites within pCH3143 that have been digested and their termini made blunt. The resulting construct is transfected into a desired cell line, for example, mouse hepatoma line Hepal-6 (ATCC #CRL1830), and selected using the drug G418, as described in Example 7. Introduction of the pKSSINhbvJR vector, or related RNA- or DNA-based alphavirus vectors, results in the production of packaged vector particles with the same hepatotropism as **HBV**.

Detailed Description Text - DETX:

Following amplification, the PCR amplicon is digested with ApaI, purified from a 1.5% agarose gel using MERMAID.TM., and ligated into pKSSINdlJRsjrc, prepared as described for **HBV**. The resulting construct is designated pKSSINmhvJR. Other alphavirus vectors (see Example 3) are readily modified in a similar manner. Packaging of vectors modified with this MHV sequence is accomplished by using expression cassettes which produce each of the required coronavirus structural proteins: nucleocapsid (N protein; Armstrong et al., NAR 11:883, 1983); membrane (M protein, Armstrong et al., Nature 308:751, 1984); and spike (S protein, Luytjes et al., Virology 161:479, 1987). Preferably, these proteins are inserted into the vector-inducible pVGELVSdl-linker plasmid (described previously in this example) and selected for with the G418 drug following transfection into the appropriate cell type. Other expression methodologies (see Example 7) may also be readily utilized. Additional coronaviruses, for example, human coronaviruses OC43 (ATCC #VR-759) and 229E (ATCC #VR-740), can be readily used in place of MHV to produce packaged recombinant alphavirus particles which show tropism for cells in the respiratory tract.

Detailed Description Text - DETX:

In each case, the packaging sequences from **HBV,** coronavirus, retrovirus, or any other virus, also may be incorporated into alphavirus vectors at locations other than those outlined above, provided the location is not present in the subgenomic transcript. For example, the next most preferable site of insertion is the carboxy-terminal region of nonstructural protein 3, which has been shown to be highly variable in both length and sequence among all alphaviruses for which sequence information is available. Further, these applications are not limited by the ability to derive the corresponding packaging cell lines, as the necessary structural proteins also may be expressed using any of the altenative approaches described in Example 8.

Detailed Description Text - DETX:

Generation of Vector Constructs which Express **HBV** Antigens for the Induction of an Immune Response

Detailed Description Text - DETX:

1. Site-Directed Mutagenesis of **HBV** E/Core Sequence Utilizing PCR

Detailed Description Text - DETX:

The second primer corresponds to the anti-sense nucleotide sequence from SK+ plasmid polylinker and contains a Cla I site 135 bp downstream of the stop codon of the **HBV** precore/core coding region.

Detailed Description Text - DETX:

The second primer sequence corresponds to the anti-sense nucleotide sequence from the SK+ plasmid polylinker and contains a Cla I site 135 bp downstream of the stop codon of the **HBV** precore/core coding region.

Detailed Description Text - DETX:

The first PCR reaction corrects the deletion in the antisense strand and the second reaction corrects the deletion in the sense strands. PCR reactions one and two correct the mutation from CC to CCA which occurs in codon 79 and a base pair substitution from TCA to TCT in codon 81. Primer 1 contains two consecutive Xho I sites 10 bp upstream of the ATG codon of **HBV** e coding region and primer 4 contains a Cla I site 135 bp downstream of the stop codon of **HBV** precore/core coding region. The products of the first and second PCR reactions are extended in a third PCR reaction to generate one complete **HBV** precore/core coding region with the correct sequence.

Detailed Description Text - DETX:

2. Isolation of **HBV** Core Sequence

Detailed Description Text - DETX:

The PCR product from the third reaction yields the correct sequence for **HBV** precore/core coding region.

Detailed Description Text - DETX:

To isolate **HBV** core coding region, a primer is designed to introduce the Xho I restriction site upstream of the ATG start codon of the core coding region, and eliminate the 29 amino acid leader sequence of the **HBV** precore coding region.

In a fourth reaction, the **HBV** core coding region is produced using the PCR product from the third reaction and the following two primers.

Detailed Description Text - DETX:

The second primer corresponds to the anti-sense nucleotide sequence for the T-3 promoter present in the SK.sup.+ HBe plasmid. The approximately 600 bp PCR product from the fourth PCR reaction contains the **HBV** core coding region and novel Xho I restriction sites at the 5' end and Cla I restriction sites at the 3' end that was present in the multicloning site of SK.sup.+ HBe plasmid.

Detailed Description Text - DETX:

Following the fourth PCR reaction, the solution is transferred into a fresh 1.5 ml microfuge tube. Fifty microliters of 3 M sodium acetate is added to this solution followed by 500 .mu.l of chloroform:isoamyl alcohol (24:1). The mixture is vortexed and then centrifuged at 14,000 rpm for 5 minutes. The aqueous phase is transferred to a fresh microfuge tube and 1.0 ml 100% EtOH is added. This solution is incubated at -20.degree. C. for 4.5 hours, and then centrifuged at 10,000 rpm for 20 minutes. The supernatant is decanted, and the pellet rinsed with 500 .mu.l of 70% EtOH. The pellet is dried by centrifugation at 10,000 rpm under vacuum and then resuspended in 10 .mu.l deionized H.sub.2 O. One microliter of the PCR product is analyzed by 1.5% agarose gel electrophoresis. The approximately 600 bp Xho I-Cla I **HBV** core PCR fragment is cloned into the Xho I-Cla I site of SK.sup.+ plasmid. This plasmid is designated SK+HBc.

Detailed Description Text - DETX:

3. Isolation of **HBV** X Antigen

Detailed Description Text - DETX:

4. Construction of Sindbis Vectors Expressing HBVE, **HBV** Core and **HBV** X

Detailed Description Text - DETX:

Construction of a Sindbis vector expressing the **HBV** core sequence is accomplished by digestion of plasmid SK+HBc (described above) with Xho I and Xba I. The HBc fragment is isolated by agarose gel electrophoresis, purified by GENECLEAN.TM. and ligated into pKSSINBV at the Xho I and Xba I sites. This Sindbis-HBc vector is designated pKSSIN-HBc.

Detailed Description Text - DETX:

Construction of a Sindbis vector expressing the **HBV**-X antigen sequence is accomplished by digesting the plasmid SK-X Ag with Xho I and Xba I to release a

cDNA fragment encoding **HBV**-X sequences. The fragment is isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and inserted into pKSSINBV, pre-treated with Xho I and Xba I enzymes. This Sindbis-**HBx** vector is designated pKSIN-**HBx**.

Detailed Description Text - DETX:

The above Sindbis **HBV** expressing vectors may also be modified to coexpress a selectable drug resistance marker dependent on the requirements of the experiment or treatment of the vector infected cells. In particular, any of the above Sindbis **HBV** expression vectors described may also be designed to coexpress G418 resistance. This is accomplished by incorporating an internal ribosomal entry site (Example 5) followed by the bacterial neomycin phosphotransferase gene placed 3' of the **HBV** coding sequences and 5' of the terminal 3' end of the vector using the multiple cloning site of the vector. These G418 resistant vector constructs can be used for selecting vector infected cells for the generation of **HBV** specific CTL targets in the following sections.

Detailed Description Text - DETX:

Cell lysates from cells infected by any of the **HBV** expressing vectors are made by washing 1.0.times.10.sup.7 cultured cells with PBS, resuspending the cells to a total volume of 600 .mu.l in PBS, and sonicating for two 5-second periods at a setting of 30 in a Branson sonicator, Model 350 (Fisher, Pittsburgh, Pa.) or by freeze thawing three times. Lysates are clarified by centrifugation at 10,000 rpm for 5 minutes.

Detailed Description Text - DETX:

As shown in FIG. 16, using these procedures approximately 100-200 ng/ml **HBV** e antigen is expressed in the cell lysates and 300-400 ng/ml **HBV** e antigen is secreted from BHK cells infected with the Sin BV HB e vector.

Detailed Description Text - DETX:

As shown in FIG. 17, using these procedures, approximately 40 ng/ml **HBV** core antigen is expressed in the cell lysates from 10.sup.6 BHK cells infected with the Sin BV HBcore. Mouse fibroblast cells infected with the recombinant HBcore Sindbis vector express 6-7 fold higher **HBV** core protein levels than the recombinant HBcore retroviral vector transduced cells (WO 93/15207). As shown in FIG. 18, using these procedures, approximately 12-14 ng/ml **HBV** core antigen is expressed in the cell lysates from 10.sup.6 L-M(TK-) cells infected with the SinBVHBcore vector as compared to the approximately 2 ng/ml **HBV** core antigen expressed from recombinant HBcore retroviral vector transducer cells.

Detailed Description Text - DETX:

Using these procedures, it can be shown that CTLs to **HBV** e antigen can be induced.

Detailed Description Text - DETX:

Lymphoblastoid cell lines (LCL) are established for each patient by infecting (transforming) their B-cells with fresh Epstein-Barr virus (EBV) taken from the supernatant of a 3-week-old culture of B95-8, EBV transformed marmoset leukocytes (ATCC CRL 1612). Three weeks after EBV-transformation, the LCL are infected with Sindbis vector expressing **HBV** core or e antigen and G418 resistance. Vector infection of LCL is accomplished by infecting LCL cells with packaged alphavirus vector particles produced from the appropriaste cell line. The culture medium consists of RPMI 1640, 20% heat inactivated fetal bovine serum (Hyclone, Logan, Utah), 5.0 mM sodium pyruvate and 5.0 mM non-essential amino acids. Infected LCL cells are selected by adding 800 .mu.g/ml G418. The Jurkat A2/K.sup.b cells (L. Sherman, Scripps Institute, San Diego, Calif.) are infected essentially as described for the infection of LCL cells.

Detailed Description Text - DETX:

Humoral immune responses in mice specific for **HBV** core and e antigens are detected by ELISA. The ELISA protocol utilizes 100 .mu.g/well of recombinant **HBV** core and recombinant **HBV** e antigen (Biogen, Geneva, Switzerland) to coat 96-well plates. Sera from mice immunized with vector expressing **HBV** core or **HBV** e antigen are then serially diluted in the antigen-coated wells and incubated for 1 to 2 hours at room temperature. After incubation, a mixture of rabbit anti-mouse IgG1, IgG2a, IgG2b, and IgG3 with equivalent titers is added to the wells. Horseradish peroxidase ("HRP")-conjugated goat anti-rabbit anti-serum is added to each well and the samples are incubated for 1 to 2 hours at room temperature. After incubation, reactivity is visualized by adding the appropriate substrate. Color will develop in wells that contain IgG antibodies specific for **HBV** core or **HBV** e antigen.

Detailed Description Text - DETX:

Antigen induced T-helper activity resulting from two or three injections of Sindbis vector expressing **HBV** core or e antigen, is measured in vitro. Specifically, splenocytes from immunized mice are restimulated in vitro at a predetermined ratio with cells expressing **HBV** core or e antigen or with cells not expressing **HBV** core or e antigen as a negative control. After five days at 37.degree. C. and 5% CO.sub.2 in RPMI 1640 culture medium containing 5% FBS, 1.0 mM sodium pyruvate and 10.sup.-5 2-mercaptoethanol, the supernatant is tested for IL-2 activity. IL-2 is secreted specifically by T-helper cells stimulated by **HBV** core or e antigen, and its activity is measured using the CTL clone, CTLL-2 (ATCC TIB 214). Briefly, the CTLL-2 clone is dependent on IL-2 for growth and win not proliferate in the absence of IL-2. CTLL-2 cells are added to serial dilutions of supernatant test samples in a 96-well plate and

incubated at 37.degree. C. and 5%, CO.sub.2 for 3 days. Subsequently, 0.5 .mu.Ci .sup.3 H-thymidine is added to the CTLL-2 cells. 0.5Ci .sup.3 H-thymidine is incorporated only if the CTLL-2 cells proliferate. After an overnight incubation, cells are harvested using a PHD cell harvester (Cambridge Technology Inc., Watertown, Mass.) and counted in a Beckman beta counter. The amount of IL-2 in a sample is determined from a standard curve generated from a recombinant IL-2 standard obtained from Boehringer Mannheim (Indianapolis, Ind.).

Detailed Description Text - DETX:

The mouse system may also be used to evaluate the induction of humoral and cell-mediated immune responses with direct administration of Sindbis vector encoding **HBV** core or e antigen. Briefly, six- to eight-week-old female C3H/He mice are injected intramuscularly (i.m.) with 0.1 ml of reconstituted (with sterile deionized, distilled water) or intraperitoneally (ip) with 1.0 ml of lyophilized **HBV** core or **HBV** e expressing Sindbis vector. Two injections are given one week apart. Seven days after the second injection, the animals are sacrificed. Chromium release CTL assays are then performed essentially as described in Example 13E 1.a

Detailed Description Text - DETX:

The data generated in the mouse system described above is used to determine the protocol of administration of vector in chimpanzees chronically infected with hepatitis B virus. Based on the induction of **HBV**-specific CTLs in mice, the subjects in chimpanzee trials will receive four doses of vector encoding core or e antigen at 7 day intervals given in two successively escalating dosage groups. Control subjects will receive a placebo comprised of formulation media. The dosage will be either 10.sup.7 or 10.sup.8 pfu given in four 1.0 ml injections i.m. on each injection day. Blood samples will be drawn on days 0, 14, 28, 42, 56, 70, and 84 in order to measure serum alanine aminotransferase (ALT) levels, the presence of hepatitis B e antigen, the presence of antibodies directed against the hepatitis B e antigen, serum **HBV** DNA levels and to assess safety and tolerability of the treatment. The hepatitis B e antigen and antibodies to HB e antigen is detected by Abbott HB e rDNA EIA kit (Abbott Laboratories Diagnostic Division, Chicago, Ill.) and the serum **HBV** DNA levels is determined by the Chiron bDNA assay. Efficacy of the induction of CTLs against hepatitis B core or e antigen can be determined as in Example 13E 1.c.

Detailed Description Text - DETX:

Based on the safety and efficacy results from the chimpanzee studies, the dosage and inoculation schedule is determined for administration of the vector to subjects in human trials. These subjects are monitored for serum ALT levels, presence of **HBV** e antigen, the presence of antibodies directed against the **HBV** e antigen and serum **HBV** DNA levels essentially as described above. Induction of human CTLs against hepatitis B core or e antigen is determined as in Example 13E 1.c.

Detailed Description Text - DETX:

G. Generation of ELVIS Vector Constructs which Express **HBV** Antigens for the Induction of an Immune Response

Detailed Description Text - DETX:

1. Construction of ELVIS Vectors Expressing HBVe-c, **HBV** Core and **HBV** X

Detailed Description Text - DETX:

Construction of an ELVIS vector expressing the **HBV** e antigen is accomplished by digesting the SK.sup.+ HB e-c plasmid with Xho I and Not I to release the cDNA fragment encoding HBVe-c sequences. The fragment is then isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and inserted into pVGELVIS-SINBV-linker vector, previously prepared by digestion with Xho I and Not I. This construct is designated pVGELVIS-HBe.

Detailed Description Text - DETX:

The HBcore PCR product described previously is digested with Xho I and Cla I, isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and ligated into SK+II (Bluescript, Stratagene, Calif.) digested with Xho I and Cla I. This construct is designated SK+HBcore. Construction of the ELVIS vector expressing the **HBV** core sequence is accomplished by digesting the SK.sup.+ HBcore plasmid with Xho I and Not I to release the cDNA fragment encoding HBVcore sequences. The fragment is then isolated by agarose gel electrophoresis, purified using GENECLEAN.TM., and inserted into pVGELVIS-SINBV-linker vector, prepared by digestion with Xho I and Not I. This construct is designated pVGELVIS-HBcore.

Detailed Description Text - DETX:

Construction of the ELVIS vector expressing the **HBV**-X antigen sequence is accomplished by digesting the plasmid SK-X Ag with Xho I and Not I to release the cDNA fragment encoding **HBV**-X sequences. The fragment is then isolated by agarose gel electrophoresis, purified using GENECLEAN, and inserted into the pVGELVIS-SINBV-linker vector, prepared by digestion with Xho I and Not I. This construct is designated pVGELVIS-**HBX**.

Detailed Description Text - DETX:

Any of the above three constructs can be used for selecting vector infected cells for the generation of **HBV** specific CTL targets in the following sections.

Detailed Description Text - DETX:

The pVGELVIS-HBe plasmid DNA is isolated and purified, and 2 ug of pVGELVIS-HBe DNA is complexed with 10 ul of LIPOFECTAMINE.TM. and transfected into 2.times.10.sup.5 BHK cells contained in 35 mM petri plates. Two days post-transfection, supernatants and whole cell lysates were collected and an ELISA assay (see below) was used to determine the amount of expressed **HBV**-e antigen.

Detailed Description Text - DETX:

As shown in FIG. 19, using these procedures, approximately 2 ng/ml **HBV** e antigen is expressed in the cell lysates and also secreted from BHK cells transfected with different clones of the pVGELVISHBe plasmid.

Detailed Description Text - DETX:

The mouse model system is also used to evaluate the induction of humoral and cell-mediated immune responses following direct administration of ELVIS vector expressing **HBV** core or e antigen. Briefly, six- to eight-week-old female Balb/c, C57B1/6, C3H/He mice (Charles River, Mass.) and HLA A2.1 transgenic mice (V. Engelhard, Charlottesville, Va.) are injected intramuscularly (i.m.) with, for example, 50 ug or greater, pVGELVIS-HBcore, pVGELVIS-HBVe or pVGELVIS-**HBX** vector DNA. Two injections are given one week apart. Seven or fourteen days after the second injection, the animals are sacrificed. Chromium release CTL assays are then performed essentially as described in Example 13E 1 .a. Detection of humoral immune responses in mice is performed essentially as described in Example 13E 2 and detection of T cell proliferation in mice is performed essentially as described in Example 13E 3.

US-PAT-NO: 6365372

DOCUMENT-IDENTIFIER: US 6365372 B1

TITLE: SNF2 related CBP activator protein (SRCAP)

DATE-ISSUED: April 2, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Chrivia; John	Kirkwood	MO	N/A	N/A
Yaciuk; Peter	Webster Groves	MO	N/A	N/A

APPL-NO: 09/ 579181

DATE FILED: May 25, 2000

PARENT-CASE:

This application claims the benefit of U.S. Provisional Application No. 60/136,620, filed May 27, 1999, incorporated in its entirety herein by reference.

US-CL-CURRENT: 435/69.1; 435/16 ; 530/350 ; 530/389.1 ; 536/23.1

ABSTRACT:

A protein, SRCAP, is provided. The protein is capable of co-activating CREB binding protein (CBP) mediated transcription, as well as activating transcription without CBP. SRCAP is a Snf2 family member. As such, it has ATPase activity. Fragments of SRCAP are also provided, as are polynucleotides encoding SRCAP and its fragments. Antibodies that bind to SRCAP are also provided. These compositions are useful for enhancing transcription in cells and patients. The compositions are also useful for reducing transcription in cells and patients.

30 Claims, 21 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 21

---------- KWIC ---------

Brief Summary Text - BSTX:

CBP is a histone acetyltransferase capable of acetylating not only histones but also several transcription factors such as GATA-1 and p53 (Boyes et al., 1998,

Nature 396, 594-598; Hung et al., 1999, Cell Biol. 19, 3496-3505; Webster et al., 1999, Mol. Cell. Biol. 19, 3485-3495). CBP also binds to several proteins that also finction as histone acetyltransferases (P/CAF, p/CIP and the p160 co-**activators such as SRC**-1).

Brief Summary Text - BSTX:

Precisely how CBP interacts with these co-activators and other cellular factors to activate transcription has not been completely elucidated. The notion that CBP interacts with a specific subset of factors at different promoters was first suggested by the work of Korzus et al., 1998, Science 279, 703-707. These authors showed that CBP in conjunction with P/CIP, **SRC-1 and P/CAF was required for activation** of transcription of a RARE reporter gene by the retinoic acid receptor, whereas only CBP, P/CAF and p/CIP were needed for activation of a CRE reporter gene by CREB and only CBP and P/CAF are required for transcription of a GAS-reporter gene by STAT-1. In addition the HAT activity of each of these co-activators was not needed at each promoter. For example, with the RARE- reporter gene, despite the fact that the pCIP-P/CAF-**SRC-1-CBP complex was needed for activation** of transcription, only the HAT activity of P/CAF was needed, whereas transcription of the CRE-reporter by the CBP-P/CAF-pCIP complex required the HAT activity of CBP not p/CAF. Thus, the specific transcription factors which CBP binds determine not only the requirement for specific co-activators but whether their HAT activity is also needed. The requirement for a specific HAT function may also be altered depending on what signaling pathways activate transcription. Xu et al. (1998, Nature 395, 301-306) have reported that forskolin activation of Pitl mediated transcription requires the HAT finction of CBP whereas insulin activation of Pitl mediated transcription does not.

Brief Summary Text - BSTX:

CBP is critical for the functioning of several viruses that impact human health. This includes human T cell lymphotrophic virus (HTLV-1), which recruits CBP to viral promoters through interaction of the viral protein TAX with CREB (Kwok et at., 1996). CBP also interacts directly with the viral transactivator protein Tat of human immunodeficiency virus type I (mHV-1) (Hottiger and Nabel, 1998, J. Virol. 72, 8252-8256). In humans, adenoviruses cause several diseases including: acute follicular conjunctivitis, pharyngoconjunctivial fever, epidermic keratoconjunctivitis, acute hemorrhagic cystitis, cervicitis, infantile diarrhea, and respiratory tract infections in children. Immune compromised individuals such as those who have undergone bone or organ transplant or who have AIDS are particularly susceptible to adenoviral caused diseases (reviewed in Chapter 65 of Zinsser, 1992, Microbiology, 20.sup.th Ed., Joklik et al., Eds., Appleton and Lange). CBP interacts with several isoforms of the adenoviral protein EIA to mediate repression or activation of transcription (Felzien et al., 1999, Mol. Cell. Biol. 19,42616). CBP also functions as a co-activator for the zta protein of Epstein-Barr virus (Zerby et al., 1999, Moll. Cell. Biol. 19, 1617-1626). Epstein-Barr virus infections have been associated with fatal lymphoproliferation in immune deficient patients and the development of Burkitt's lymphoma (reviewed in Zinsser Microbiology, 1992, supra). CBP has

also been indirectly implicated in the transcriptional regulation of other viruses through interaction with CREB. For example, CREB binds to the enhancer of the IE1/2 gene of human cytomegalovirus (Lang et al., 1992, Nucl. Acids Res. 20, 3287-3295), an important pathogen in immunosuppressed patients such as transplant recipients and AIDS patients (Drew, 1988, J. Infect. Dis. 158, 449456). CREB also binds the hepatitis B virus (**HBV**) enhancer element when complexed with the **HBV** protein, pX (Maguire et al., 1991, Science 252, 842-844). A possible role of CBP in some forms of cancer is suggested by the observation that CBP is part of a multi-subunit complex with the breast cancer tumor suppressor BRCA1 and RNA polymerase II (Neish et al., 1998, Nucl. Acids Res. 26, 847-853). In some forms of acute myeloid leukemia a t(7;11)(p15: p15) translocation results in the NUP98-HOXA9 fusion protein, which is a strong transcriptional activator that uses CBP as a co-activator (Kasper et al., 1999).

Detailed Description Text - DETX:

SRCAP would also be expected to affect several aspects of virus infection. Examples include the interaction of the HTILV-1 protein TAX with CREB; the interaction of CBP with the HIV-1 Tat protein; various effects of adenovirus infection due to the interaction of CBP with the adenovirus E1A protein; various effects of Epstein-Barr virus due to the interaction of CBP with the viral zta protein; various effects of cytomegalovirus due to the interaction of CREB with the enhancer of the viral IE1/2 gene; various effects of hepatitis B virus (**HBV**) due to the interaction of CREB with the viral protein pX; and various effects of hepatitis C virus due to the interaction of SRCAP with the viral protein NS5A (see Example 2).

Detailed Description Text - DETX:

The present invention also provides methods of treating a patient having a disease involving a function affected by SRCAP. The methods comprise treating a cell of the patient with an amount of a polypeptide, polynucleotide, or antibody composition of the invention sufficient to modulate transcription of the cell. As described supra in the context of the cell treatment methods, the treatment can be directed toward increasing the presence of SRCAP (e.g., by SRCAP or SRCAP fragment polypeptide or polynucleotide) or decreasing the presence of SRCAP (e.g., by antisense, ribozyme or antibody treatment). In particular embodiments, the function is insufficient transcription of a gene selected from the group consisting of a gene mediated by CBP co-activation, a DEAD box RNA dependent helicase (see Example 3), adenoviral DBP protein, .beta.-actin, and a nuclear receptor. An example of a nuclear receptor is the glucocorticoid receptor (GR). In these embodiments, the cell is treated with a SRCAP polypeptide or SRCAP polynucleotide, or fragments thereof. In other embodiments, the function is undesired effects caused by HCV NS5A, HTLV-1 TAX protein, HIV-1 Tat protein, adenovirus EIA protein, Epstein-Barr virus zta protein, cytomegalovirus IE1/2 enhancer, hepatitis B virus (**HBV**) pX protein. As previously discussed, when reduction of transcription is desired, the cell may be treated with SRCAP antisense or ribozyme preparations, or with anti-SRCAP antibodies.